Apoptosis, Necrosis, and Autophagy

C1 Harnessing the pro-inflammatory tumor microenvironment of castrate-resistant prostate cancer to promote apoptosis. Christopher McCann, Nyree Crawford, David Waugh, Daniel Longley. Queen's University Belfast, Belfast, United Kingdom.

Introduction: Castrate-Resistant Prostate Cancer (CRPC) represents a highly aggressive, currently incurable disease state. Overexpression of anti-apoptotic proteins including FLIP (FLICE-inhibitory protein) and inhibitors of apoptosis proteins (cIAP-1, cIAP-2 and XIAP) in prostate cancer have been associated with progression to castrate-resistant disease. These proteins are known to contribute to resistance to standard-of-care therapies and aid cancer progression through their anti-apoptotic activity. SMAC mimetics are small molecule compounds which mimic the activity of the endogenous inhibitor of IAPs known as SMAC (Second Mitochondrial-derived Activator of Caspase). SMAC mimetic activity is dependent on TNFα signalling and has also been shown to induce apoptosis in response to TRAIL therapy. Both TRAIL and TNFα are derived from immune cells that are frequently present in the tumour microenvironment, making SMAC mimetic therapy an attractive strategy for treating cancers associated with pro-inflammatory immune infiltrates.

Methods: A panel of prostate cancer cell lines were treated with SMAC mimetic alone, or in combination with exogenous TNFα or TRAIL. Cell viability was determined by MTT assay, and apoptosis was assessed using Western blotting and AnnexinV/PI flow cytometry. siRNA and Histone Deacetylase Inhibitor (HDACi) therapy were employed to reduce FLIP expression. The addition of exogenous TNFα, TRAIL or macrophage-conditioned media was used to model the pro-inflammatory microenvironment.

Results: Prostate cancer cells were found to be resistant to SMAC mimetic therapy alone and in the presence of TNFα or TRAIL. FLIP expression was found to be a resistance mechanism to SMAC-mimetic treatment. Depletion of FLIP expression by siRNA or HDACi therapy resulted in an increased sensitivity to TNFα, TRAIL and macrophage conditioned media alone and in combination with SMAC mimetics. Targeting FLIP alone may therefore be a more effective means of harnessing the pro-inflammatory microenvironment. Moreover, the apoptosis induced in the presence of macrophage conditioned media by SMAC mimetics in FLIP-depleted cells was demonstrated to be TNFα-dependent using TNFα neutralising antibodies.

Conclusion: Prostate cancer cell lines are inherently resistant to SMAC mimetic therapy even in the presence of TNFα or TRAIL. However, dual targeting of FLIP and IAPs sensitises CRPC cell lines to microenvironment-derived TNFα. Combined targeting of IAPs and FLIP, or single targeting of FLIP, may be an effective means of treating pro-inflammatory prostate cancer.

C2 Clinical safety, pharmacokinetics, and early evidence of activity of the oral IAPs inhibitor Debio 1143 in combination with carboplatin and paclitaxel: a phase I b study. Isabelle Ray-Coquard1, Christophe Le Tourneau2, Nicolas Isambert3, Carlos A. Gomez-Roca4, Philippe Cassier1, Marie Paule Sablin2, Bruno Gavillet5, Daniela Purcea6, Elisabeth Routis7, Claudia Schusterbauer8, Claudio Zanna8, Pierre Fumoleau2, Jean-Pierre Delord4. 1Centre Léon Berard, Département de médecine carcinologique, Lyon, France; 2Institut Curie, Département d’oncologie médicale, Paris, France; 3Centre Georges-Francois Leclerc, Service d’oncologie médicale, Dijon, France; 4IUCT Oncopole, Oncologie médicale, Toulouse, France; 5Debiopharm International SA, Lausanne, Switzerland.

Background: Resistance to apoptosis is a typical hallmark of cancer. Inhibitor of Apoptosis Proteins (IAPs) block caspase activation, modulate NF-kB signaling pathways, and are involved in resistance to standard chemo and radiation therapies. As such, IAPs antagonism represents an attractive target for therapeutic
intervention. Debio 1143 is a potent orally-available monovalent SMAC mimic antagonist of IAPs currently in clinical development. A previous phase I study showed Debio 1143 was well tolerated up to 900 mg QD as a single agent, with strong evidence of pharmacodynamic (PD) activity and appropriate pharmacokinetic (PK) disposition at doses ≥ 120 mg. [1] This Phase I study defined the dose-limiting toxicities (DLTs), maximum tolerated dose (MTD), safety, PK, and PD of Debio 1143 in combination with carboplatin and paclitaxel.

Methods: Treatment-naïve and previously treated patients with squamous non-small cell lung cancer (NSCLC), and previously treated patients with triple negative breast cancer (TNBC) and platinum-resistant epithelial ovarian cancer (EOC) were treated with carboplatin (AUC 6) and paclitaxel 175 mg/m² on day 1 of every 21-day-cycle up to a maximum of 6 cycles, combined with escalating doses of Debio 1143, administered once daily on days 1-5 of every 21-day cycle. The starting dose of Debio 1143 was 200 mg QD.

Results: Thirty-one patients were included in the study. Four patients are still on treatment. Hematological DLTs were observed in 2/4 patients in the first two dose levels despite the reduction of Debio 1143 dose to 100 mg. PK data suggested a PK interaction between Debio 1143 and paclitaxel. The study protocol was amended to reduce the doses of carboplatin to AUC 5 and paclitaxel to 135 mg/m². Afterwards, patients received Debio 1143 doses of 100 mg (n=2), 125 mg (n=2), 175 mg (n=2), 200 mg (n=7), 225 mg (n=5), 250 mg (n=9). DLTs were febrile neutropenia (n=2) and G3 ALT increase (n=1). The MTD of Debio 1143 to be combined with carboplatin (AUC 5) and paclitaxel (135 mg/m²) is 250 mg QD. Most common treatment-related AEs reported in ≥ 15% of patients were asthenia (n=13), decreased appetite (n=11), diarrhea (n=9), neutropenia (n=9), thrombocytopenia (n=8), anemia (n=8), nausea (n=7), fatigue (n=7), vomiting (n=4), AST increase (n=4), hypomagnesaemia (n=4), myalgia (n=4), and epistaxis (n=4). Paclitaxel exposure in patients dosed at 135 mg/m² in combination with Debio 1143 was similar to historical control dosed at 175 mg/m² due to a 25 to 40% reduction in clearance. Preliminary evaluation of PD endpoints confirmed Debio 1143-induced cIAP1 degradation in PBMCs at doses starting from 100mg. In line with the expected mechanism of action, modulation of serum PD markers of NF-kB signaling pathways and epithelial apoptosis was also observed during treatment. Twenty-three patients were evaluable for response. Partial responses by RECIST have been observed in 7 patients with EOC (6/17) and TNBC (1/5).

Conclusions: The combination of Debio 1143 with carboplatin and paclitaxel is well tolerated, with significant PD activity and hints of activity. The clinical PK interaction between Debio 1143 and paclitaxel is well-controlled and managed in the patients. These findings support a further Phase II program.


C3 Activating necroptosis in acute myeloid leukemia through inhibition of PKC, calmodulin, and HOX/PBX dimerization . Raed Alharbi1, Ruth Pettengell2, Mohamed El-Tanani3, Hardev S. Panda4, Richard Morgan2. 1University of Surrey, Guildford, United Kingdom; 2University of London, London, United Kingdom; 5University of Bradford, Bradford, United Kingdom.

The molecular mechanisms underlying the pathogenesis of acute myeloid leukemia (AML) have been extensively studied, and are known to involve members of the HOX family of transcription factors, both as partners in chimeric fusion proteins, and also in their wild type form. However, the role of HOX proteins in the survival of AML cells has proved difficult to assess as many have redundant functions, which makes a conventional knock down experiment difficult to interpret. An alternative strategy to targeting HOX proteins is to inhibit their interaction with the PBX co-factor, which can be achieved using a short, cell-penetrating
peptide (HXR9) that mimics the conserved hexapeptide in HOX proteins responsible for PBX binding. HXR9 has been shown to induce apoptosis in malignant B cells, and a number of AML cell lines.

In order to evaluate the molecular mechanisms underlying the cytotoxicity of HXR9 in AML cells, we determined the sensitivity of a number of AML-derived cell lines. Three of these (KG1, HEL 92.1.7, and HL-60) are derived from primary AML, and two from secondary AML (KU812F, and K562). The IC50s of cell killing by HXR9, as determined using a lactose dehydrogenase (LDH) assay, were 4.5, 6.1, 16.9, 9.1, and 10.4 μM, respectively. The K562 cells were also sensitive to HXR9-mediated killing in vivo, as administration of HXR9 could significantly inhibit the growth of K562 flank tumors in a mouse xenograft model.

We investigated whether these cells underwent apoptosis after HXR9 treatment. There were no definitive changes associated with apoptosis including caspase-3 activation, PARP cleavage, or nuclear fragmentation. Furthermore, cell death was not dependent on ATP, and could not be reversed by the pan-caspase inhibitor z-VAD-FMK. We therefore explored the possibility of necrotic cell death. Neither K562 nor HL-60 cells could be rescued using CsA, an inhibitor of mitochondrial necrosis that targets the CypD protein. However, inhibition of the RIP1 kinase using its inhibitor Nec-1 resulted in a significant rescue of K562 and HL-60 cells from HXR9-mediated cytotoxicity. RIP1 is a central component of the necroptosis pathway, suggesting that this might play a key role in HXR9-induced cell death. We also explored further molecular pathways that might influence necroptosis. Inhibition of signaling through p38, JNK, and MEK/ERK had no effect on HXR9 cytotoxicity, nor did the inhibition of the p53 tumor suppressor protein, although HXR9 treatment of both K562 and HL-60 cells resulted in a significant increase in expression of the p21 tumor suppressor gene. Furthermore, inhibition of both protein kinase C (PKC) and calmodulin significantly sensitized cells to HXR9.

Taken together, our findings point to a mechanism of HXR9-mediated cell death that depends not on apoptosis, but instead on necroptosis, and which can be blocked by PKC signaling. Necroptosis is considered to be a regulated form of necrosis that in some respects parallels apoptosis, as it can be triggered by the same external stimuli. Its interaction with PKC and calmodulin-mediated signaling points to possible synergistic approaches when targeting AML.

C4 Development and pre-clinical assessment of a first-in-class small molecule inhibitor of FLIP for treatment of NSCLC. Joanna Majkut1, Catherine Higgins1, Adnan Malik1, Zsusannah Nemeth1, Peter Blurton2, Ray J. Boffey3, Trevor R. Perrior2, David Haigh1, Timothy Harrison1, Daniel B. Longley1. 1Queen's University Belfast, Belfast, United Kingdom;2Domainex Ltd., Cambridge, United Kingdom.

Background: Evasion of cell death is a major cause of resistance to cancer therapy, making proteins that regulate cell death clinically relevant therapeutic targets. The anti-apoptotic protein FLIP is frequently overexpressed in a number of cancers, including non-small cell lung cancer (NSCLC), and has been shown by us and others to be a major mediator of drug resistance. FLIP and procaspase-8 form complexes with the adaptor protein FADD in response to a variety of clinically relevant stimuli, including ligation of death receptors, such as TRAIL-R1 and R2, and treatment with chemotherapeutic agents. In these complexes, FLIP modulates the activation of procaspase-8, and thereby apoptosis and necroptosis - two major cell death mechanisms. We recently reported that there are important differences between FLIP and procaspase-8 in terms of both their binding affinities and preferred modes of interaction with FADD that are potentially therapeutically exploitable [1]. We now report our subsequent work leading to the development and pre-clinical characterisation of first-in-class inhibitors of FLIP.

Methods: Molecular modelling of the FLIP-FADD complex; virtual small molecule library screening; cell-free screening assays; cell-based activity assays; biophysical binding assays; in vivo anti-tumor studies.
Results: Molecular modelling of the FLIP-FADD complex identified a putative drug-binding pocket on FLIP against which a virtual small-molecule screen was carried out. Subsequent biochemical screening of selected compounds using a FLIP-FADD protein-protein interaction assay identified hits with on-target activity. Medicinal chemistry optimisation of these hits afforded lead and back-up series with nanomolar activity in cell-based assays (i.e. caspase activation, cell death and cell survival), which is in line with their binding affinity in an orthogonal biophysical assay (isothermal calorimetry). The pro-apoptotic effects of these FLIP inhibitors were enhanced upon addition of death ligands, such as TRAIL, and lead-molecules have been shown to potentiate the effects of the standard-of-care chemotherapeutic cisplatin. FLIP overexpression and procaspase-8 depletion abrogated the effects of these novel inhibitors consistent with the expected mechanism-of-action. Lead molecules have been identified with ADME profiles suitable for in vivo evaluation. Using these compounds, single-agent anti-tumor effects have been demonstrated in NSCLC xenograft models.

Conclusions: The novel first-in-class inhibitors of FLIP developed in this study have the potential for broad application in treatment of NSCLC, either as monotherapy or in combination with other agents.

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C5 FLIP protein-protein interaction inhibitors enhance sensitivity of colorectal cancer cells to chemotherapy and TRAIL. Jennifer P. Fox1, Joanna Majkut1, Catherine Higgins1, Zsuzsanna Nemeth1, Adnan Malik1, Christopher J. Scott1, Peter Blurton2, Ray J. Boffey2, Trevor R. Perrior2, Timothy Harrison1, Daniel B. Longley1.1Queens University Belfast, Belfast, United Kingdom; 2Domainex Ltd, Cambridge, United Kingdom.

Background: Colorectal Cancer (CRC) is the second most common cause of cancer death, with 40% of patients with this disease obtaining no benefit from current chemotherapy. Novel therapeutic strategies are needed to improve CRC patient response rates and survival. FLIP is an inhibitor of the extrinsic apoptotic pathway that binds to FADD at death-inducing signalling complexes (DISCs), such as those formed by the TNF-α-related apoptosis inducing ligand (TRAIL) receptors TRAIL-R1 and TRAIL-R2, thereby blocking homodimerization and activation of procaspase-8 and inhibiting apoptosis induction. We previously reported that FLIP blocks apoptosis induced by TRAIL and standard-of-care chemotherapeutics (5-Fluorouracil, oxaliplatin and SN38) in CRC models. Moreover, FLIP is frequently overexpressed in CRC and its overexpression correlates with poor prognosis. Subsequently, we have developed novel small molecule inhibitors that target FLIP’s critical protein-protein interactions, preventing its interaction with FADD and therefore promoting activation of caspase-8 and apoptosis induction.

Methods: A DISC recruitment assay was used to assess levels of FLIP at the TRAIL-R2 DISC. Caspase activity, cell viability and apoptosis induction were assessed in CRC models treated with FLIP inhibitors alone and in combination with TRAIL or standard-of-care chemotherapeutics. Mechanism-of-action was assessed using caspase-8-targeted siRNA and FLIP overexpressing models.

Results: Using CRC cell line models, it was demonstrated that FLIP recruitment to the DISC is inhibited by FLIP inhibitors from the lead series. As a result, TRAIL-induced caspase-8 and caspase-3/7 activity were enhanced and increased levels of apoptosis cells were observed. Decreased cell viability was observed that was proportionate to the levels of apoptosis induced. Cell death triggered by FLIP inhibitors was shown to
be caspase-8-dependent consistent with the expected mechanism-of-action. Importantly, inhibitors of FLIP also enhanced apoptosis induction in response to 5-Fluorouracil, oxaliplatin and SN38.

Conclusion: We have developed inhibitors of FLIP that decrease its recruitment to the TRAIL-R2 DISC and increase TRAIL-induced caspase activation and apoptosis. Moreover, these inhibitors synergise with 5-Fluorouracil, oxaliplatin and SN38, suggesting that this novel class of agents has therapeutic potential in CRC when used in conjunction with standard-of-care chemotherapeutic agents.

Acknowledgements: This work was supported by a Seeding Drug Discovery award from the Wellcome Trust (reference: 099470).

C6 Antiapoptotic defense evolution in cancer: Novel therapeutic strategies to restore cell death using dynamic BH3 profiling. Joan Montero, Dorota E. Sadowicz, Rizwan Haq*, Anthony Letai*. Dana-Farber Cancer Institute, Boston, MA. *authors contributed equally

Background: There is a lack of effective predictive biomarkers to efficiently assess the optimal treatment for each patient and overcome resistance. When effective death signaling is initiated by a targeted therapy, an increase in mitochondrial apoptotic sensitivity (or ‘priming for death’) can be observed within hours with Dynamic BH3 Profiling (DBP). Most chemotherapeutic agents kill via the mitochondrial pathway of apoptosis, but unfortunately tumors frequently are able to survive adapting their antiapoptotic strategy. By using different BH3 peptides we can determine the tumor’s defense adaptation over time and find novel ways to rationally develop combination therapies and restore apoptotic cell death.

Hypothesis: Analyzing the tumor’s antipoptotic defense over time with DBP we can predict how the tumor will adapt to the first-line therapy and restore cell death using BH3 mimetics.

Results: Our first set of experiments consisted in characterizing the tumor’s adaptation to therapy over time. When effective death signaling is initiated by a targeted therapy, an increase in mitochondrial apoptotic sensitivity (or ‘priming for death’) can be observed within hours with Dynamic BH3 Profiling (DBP). Most chemotherapeutic agents kill via the mitochondrial pathway of apoptosis, but unfortunately tumors frequently are able to survive adapting their antiapoptotic strategy. By using different BH3 peptides we can determine the tumor’s defense adaptation over time and find novel ways to rationally develop combination therapies and restore apoptotic cell death.

Exploiting DBP’s capacity to measure changes in priming without the requirement for prolonged ex vivo culture, we assessed if this antipoptotic evolution was also present in primary melanoma samples. We tested combinations of targeted therapies with BH3 mimetics to incorporate antiapoptotic targets to cancer treatment.

Conclusions: Our cell line and clinical experiments demonstrate the potential for Dynamic BH3 profiling to be used as a powerful real-time tool to predict chemotherapy response and evaluate induced dependencies in relapsed patients. By studying combinations of targeted agents with BH3 mimetics we aim to improve cancer treatment.
C7  Mechanistic studying involving the combined effect of Cyclooxygenase-2 inhibitor and Cholecystokinin -2 Receptor antagonist on Pancreatic cancer cell. Manisha Sikka, Madhu Chopra. Dr.B.R. Ambedkar Centre for Biomedical Research, Delhi, University of Delhi, Delhi, India.

Introduction: Pancreatic ductal adenocarcinoma is the fourth leading cause of cancer death in United States. Due to poor prognosis and increasing incidences, over 80% of the patient are given palliative chemotherapy with combination drugs. Despite decades of efforts, the survival rate still remains 3-6%. Recent studies have shown an association between the gastrointestinal hormone gastrin, the expression of Cholecystokinin -B/gastrin receptor and an increased generation of COX-2 in colon-carcinoma cells and progression of gastric cancer, suggesting that gastrin has direct stimulating potencies. However, it remains unknown whether the combination of Cholecystokinin -2 (CCK-2) receptor antagonist plus COX-2 inhibitor exerts synergistic antitumor effects on human gastric cancer. Therefore, for a better treatment and understanding the underlying mechanism that leads to pancreatic adenocarcinoma initiation, we further investigated the combined effect of Cyclooxygenase (COX-2) inhibitor and Cholecystokinin receptor -2 (CCK-2) antagonist on pancreatic cancer cells. Thus it is essential to recognize more effective targets/ receptors for treatment of pancreatic cancer.

Materials and Methods: Antiproliferative effects were carried out using Cell Proliferation Kinetics, Morphological analysis and MTT Assay. Apoptotic activity was confirmed by DNA Fragmentation, Western Blot analysis and Cell cycle analysis.

Result and Discussion: To study the combinatorial effect of COX-2 inhibitor and Cholecystokinin antagonist we successfully purified Etoricoxib using HPLC and further characterized by spectroscopic techniques. The anti-proliferative activity of Etoricoxib was checked on various cell lines and it induced proliferation arrest in Miapaca-2, Panc-I, BXPC-3 and ASPC-1 both in time and concentration dependent manner. Combinatorial studies was carried out on Pancreatic cancer cells using cytotoxicity and apoptotic techniques. It is thus, a probable insight in stating that it has lesser side effects and thus have selectivity for cancer cell toxicity.

Conclusion: The combined effect of Cyclooxygenase-2 inhibitor and Cholecystokinin-2 Receptor antagonists was calculated. The results has indicated that the combination therapy of COX-2 selective inhibitors may be the potential chemotherapeutic strategy for cancer prevention. In the present study, human pancreatic cancer cell lines, in which the CCK-2 receptor and COX-2 were expressed, was applied to examine whether blockade of the CCK-2 receptor and COX-2 exerts synergistic anti-tumor effects on human pancreatic cancer in vitro. In conclusion, both Etoricoxib and YM-022 had growth inhibitory and apoptosis inductive effects on the pancreatic cancer cells through down regulation of Bcl-2 with simultaneously up-regulation of BAX expression which suggest that COX-2 inhibition is new molecular targets for effective therapy against pancreatic cancer.

C8  Targeting MCL1-dependent cancers with SF3B splicing modulators. Daniel Aird, Ermina Pazolli, Craig Furman, Linda Lee, Kaiko Kuni, Eun Sun Park, Craig Karr, Betty Chan, Michelle Aicher, Silvia Buonomici, John Yuan Wang, Jacob Feala, Lihua Yu, Markus Warmuth, Peter Smith, Peter Fekkes, Ping Zhu, Baudouin Gerard, Yoshiharu Mizui, Laura Corson. H3 Biomedicine Inc, Cambridge, MA.

Myeloid cell leukemia 1 (MCL1) is a member of the BCL2 family of proteins governing the apoptosis pathway and is one of the most frequently amplified genes in cancer. MCL1 overexpression often results in dependence on MCL1 for survival and is linked to resistance to anticancer therapies. However, the development of direct MCL1 inhibitors has proven challenging and new modalities for targeting MCL1 are required. Alternative splicing of MCL1 converts the anti-apoptotic MCL1 long (MCL1L) isoform to the BH3-
only MCL1 short (MCL1S) isoform, which has been reported to be pro-apoptotic. Thus, changing MCL1 isoform levels through modulation of RNA splicing may represent an attractive approach to targeting MCL1-amplified cancers. To this end, we tested a collection of small molecule SF3B modulators that impact RNA splicing on MCL1-dependent and MCL1-independent NSCLC cell lines.

SF3B modulators induced rapid downregulation of the long form and upregulation of the short- and intron-containing form of MCL1 across models; however, apoptosis was only observed in MCL1-dependent cells. Importantly, SF3B modulators preferentially killed MCL1-dependent cell lines and sensitivity correlated with MCL1 amplification. To dissect the mechanism of SF3B modulator-induced cytotoxicity, we overexpressed either the cDNA for the BH3-only short isoform or the full length isoform of MCL1. Surprisingly, overexpression of MCL1S cDNA had no significant effect on cells by itself and did not sensitize cells to SF3B modulator cytotoxicity. Conversely, MCL1L-specific shRNA knockdown was sufficient to kill MCL1-dependent cells and SF3B modulator cytotoxicity was rescued by expression of MCL1L cDNA. Together, these results argue that MCL1L modulation and not MCL1S upregulation is the effector of SF3B modulator cytotoxicity. In immunocompromised mice bearing MCL1-dependent xenograft models, SF3B1 modulator treatment resulted in significant downregulation of MCL1 levels accompanied by induction of apoptosis and robust efficacy at well-tolerated doses. Moreover, MCL1L cDNA expression in MCL1-dependent models rescued apoptosis induced by SF3B1 modulator treatment.

These studies provide proof-of-concept that splicing modulation is an effective strategy for targeting cancers dependent on MCL1.

C9 Pancreatic tumor growth suppression through autophagy mediated apoptosis. Alok Ranjan, Sanjay Srivastva. Texas Tech University Health Science Center, Amarillo, TX.

Pancreatic cancer is amongst the most lethal solid tumor claiming thousands of lives each year. Pancreatic tumors exhibit enhanced autophagy as compared to any other cancer. Elevated autophagy in pancreatic tumors is responsible for resistance to therapy. In this study, we evaluated the effect of penfluridol, which is a first generation antipsychotic drug against pancreatic cancer. Penfluridol treatment inhibited the growth of Panc-1, BxPC-3 and AsPC-1, pancreatic cancer cells in a concentration-dependent manner with IC50 ranging between 4-6 \( \mu \)M after 24h of treatment and induced apoptosis. Significant autophagy was induced by penfluridol treatment in pancreatic cancer cells as evaluated by acridine orange assay. Western blot analysis of Panc-1, BxPC-3 and AsPC-1 cells treated with penfluridol exhibited up-regulation of autophagy markers like LC3B and p62. Microscopic evaluation of punctate LC3B and autophagosomes staining by acridine orange in pancreatic cancer cells by penfluridol treatment further confirmed autophagy induction. Interestingly, inhibiting autophagy by chloroquine, bafilomycin, 3 methyl adenine or LC3B siRNA, significantly blocked penfluridol induced apoptosis suggesting that autophagy lead to apoptosis in our model. Efficacy of penfluridol was confirmed in an in vivo model. AsPC-1 and BxPC-3 cells were implanted subcutaneously separately in athymic nude mice and once the tumor size reached 100 mm³, treatment group received 10mg/kg penfluridol everyday by oral gavage, with or without 50 mg/kg chloroquine, a well-known inhibitor of autophagy. Penfluridol treatment suppressed the growth of BxPC-3 tumors by 52% at day 27 as compared to only 17% when treated in combination with chloroquine. Similarly, penfluridol suppressed the growth of AsPC-1 tumors by 40% versus 11% when given in combination with chloroquine. To further confirm the anti-tumor effects of penfluridol, Panc-1 luc cells were implanted orthotopically in the pancreas and the mice were treated with 10 mg/kg penfluridol everyday by oral gavage after 7 days of tumor implantation. Our results showed that penfluridol treatment substantially suppressed the growth of pancreatic tumors. Immunohistochemical analysis of tumor tissue and western blot analysis of tumor lysate
indicated upregulation of p62 and LC3B as well as cleavage of caspase 3 confirming our in vitro findings. Since penfluridol is already in clinic, we expect that any positive findings from our study will accelerate its clinical development to treat pancreatic cancer. [Supported in part by R01 grant CA129038, awarded by National Cancer Institute, NIH]

Biomarkers

C10 Quantitative proteomics of formalin-fixed paraffin-embedded, primary triple-negative breast cancer tissues of patients who experienced distant metastasis or no recurrence. Martin H. Pedersen¹, Brian L. Hood², Thomas P. Conrads², Henrik J. Ditzel¹, Rikke Leth-Larsen¹. ¹Institute for Molecular Medicine, Odense, Denmark; ²Women's Health Integrated Research Center at Inova Health System, Annandale, VA.

Triple-negative breast cancer (TNBC) represents a major subtype of breast cancer (BC) and no biomarkers or targeted treatments are available. Retrospective proteomic analysis of formalin-fixed paraffin-embedded (FFPE) BC tissue has the potential to identify novel biomarkers and pathways associated with disease recurrence in TNBC, since sample data can be coupled with clinical data, including treatment and patient outcome. Primary TNBC tissue of patients who experienced distant metastasis or no recurrence within a 10 year follow-up period was retrieved to investigate whether proteomics using liquid chromatography tandem mass spectrometry (LC-MS/MS) can identify proteins and pathways associated with their metastatic potential.

FFPE material on PEN-membrane slides were deparaffinized according to standard procedure with xylene/ethanol and stained with hematoxylin and eosin. Cancer cells were dissected by UV laser capture microdissection (LCM) and subjected to protein extraction in triethylammonium bicarbonate/acetonitrile buffer by heat-induced antigen retrieval. Extracted proteins were trypsinized and generated peptides were isobarically labelled with 6-plex tandem mass tags, combined 1:1 and subjected to hydrophilic interaction liquid chromatography LC-MS/MS.

Preliminary experiments identified >500 unique proteins from BC FFPE tissue, verifying a robust setup. Cancer cells from primary TNBC FFPE tissues of 15 patients who experienced distant metastatic and 20 patients who did not have recurrence within ten years were isolated by UV-LCM and analyzed according to the above setup. The patients were stratified into four working groups based upon their menopausal status (pre or post) and their recurrence (recurrence or no-recurrence) outcome. The median aged varied from 42.2 to 63.4 years between the four groups with most tumors being diagnosed as T1 or T2 (TNM classification system). All tumors were grade 2 or higher at diagnosis, with no distant metastasis present prior to removal of the primary tumor. We identified 2564 high-confident proteins across the 35 patient samples with ≥2 unique peptides. Statistical analysis was done in RStudio and GproX and revealed distinct molecular profiles capable of separating metastatic from non-metastatic patients with a p-value ≤0.05. Furthermore three proteins were found to be differentially regulated between the two groups with a p-value <0.001 making these highly interesting targets in the prevention of metastatic lesions in TNBC.

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Background: EGFR inhibitors, erlotinib and gefitinib, cause tumor shrinkage in approximately 70% of non-small cell lung cancer (NSCLC) patients with activating mutations in EGFR, but may also be efficient in a subset of patients with EGFR wildtype, as indicated by a retrospective analysis of the BR.21 trial. However, there is currently a lack of biomarkers predictive for outcome of EGFR inhibitor-treated NSCLC patients with EGFR wildtype. The identification of such biomarkers was the aim of this study.

Materials and methods: Formalin-fixed paraffin-embedded tissue blocks from 50 NSCLC patients with EGFR wildtype, who received treatment with erlotinib/gefitinib were included and stratified according to progression-free-survival (PFS). Twelve patients experienced no progression for >6 months (Good Response Group), while 38 patients experienced progression in <6 months (Poor Response Group). From formalin-fixed paraffin-embedded surgical resections, areas of high tumor content were isolated; peptides were extracted in triethyl ammonium bicarbonate/acetonitrile buffer by heat-induced antigen retrieval, and then fractionated by hydrophilic-interaction liquid chromatography (HILIC) followed by quantification and identification by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Samples were analyzed in technical duplicates, and only proteins identified with minimum 2 unique peptides were used for further analysis.

Results: The patient cohort represented 20 men and 30 women. Two were non-smokers, 45 were smokers/former smokers and three were unknown. 42 exhibited adenocarcinoma histology and eight exhibited bronchioalveolar carcinoma histology. The majority of patients were treated with erlotinib (n=47), while three received gefitinib. Mean PFS of patients in the Good Response Group was 455 days, while patients in the Poor Response Group had a mean PFS of 66 days, indicating that there are indeed patients with EGFR wildtype who benefit from EGFR inhibitors. From the quantitative LC-MS/MS analysis, we identified and quantified 3152 high-confident proteins across the 50 patients with ≥2 unique peptides. Softwares R and GProX were used for statistical analysis. Data was quantile normalized and revealed 30 proteins which were found to be differentially expressed between the two groups with a p-value <0.01. Among the most differentially expressed proteins, we found proteins involved with cytoskeletal rearrangement, intracellular transport, protein translation, transcription regulators and kinases.

Conclusions: From the PFS analysis of our patient cohort, it is clear that patients exhibiting EGFR wildtype may also benefit from treatment with EGFR inhibitors. Using unbiased mass spectrometry, we identified 30 differentially expressed proteins with the potential to predict outcome of EGFR inhibitor-treated NSCLC patients with EGFR wildtype.
C12 Identification of biomarkers and pathways associated with response to the DOT1L inhibitor Pinometostat (EPZ-5676) in MLL-r leukemia. Scott Daigle1, Alice McDonald1, Ty M. Thomson2, David A. Drubin3, Michael Maria4, A. Carson5, Brad Patay6, Jeff Keats7, Christine Klaus1, Alejandra Raimondi1, G. Garcia-Manero4, D. A. Rizzieri5, Raoul Tibes6, Jesus Berdeja7, Eytan M. Stein8, Blythe Thomson1, Stephen J. Blakemore1. 1Epizyme, Cambridge, MA; 2Selventa, Cambridge, MA; 3Genection Inc., San Diego, CA; 4Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX; 5Cellular Therapy, Duke Univ. Med. Ctr., Durham, NC; 6Mayo Clinic Arizona, Scottsdale, AZ; 7Sarah Cannon Research Institute, Nashville, TN; 8Memorial Sloan Kettering Cancer Center, New York, NY.

Pinometostat is a highly selective first in class DOT1L inhibitor currently in Phase 1 clinical trials in adult and pediatric leukemia patients (pts) with MLL rearrangements (MLL-r or MLL-PTD). Preliminary results of the adult trial have demonstrated clinical activity including complete remissions in a subset of patients (Stein, 2014). Investigation and identification of candidate molecular correlates of pinometostat response in both pt samples and cell lines are reported.

RNA and DNA were isolated from PBMCs and/or bone marrow collected prior to treatment from 18 pts enrolled in the adult pinometostat Phase 1 study (CT.gov: NCT01684150), at the following doses 24 (n =2), 36 (n = 3), 54 (n = 6), 80 (n = 3) and 90 mg/m2/day doses (n = 4). mRNA transcript abundance was assessed using whole genome RNASeq and DNA variants were determined using a 194 gene panel, MyAML (Genection Inc.). Correlations of transcript abundance and DNA variants detected with categorical (responder = CR or PR [n=3], or no response [n=16]) and continuous response parameters (time on study [TOS], mean = 59 days: range = 8-196 days) were performed. For cell lines, whole genome RNASeq data was generated from 14 cell lines (MLL-r or MLL-PTD) with a range of in vitro sensitivity to pinometostat (cell proliferation IC50 2 nM to > 10 μM) and RNA transcripts identified as correlated with IC50 were submitted for pathway analysis.

Univariate analyses revealed no DNA variants to be associated with either response category (FDR adjusted P < 0.05), however, a statistical association (unadjusted P = 0.05) was identified between increased TOS and those pts harboring t(11;19). Indicating that specific MLL fusion partners may elicit differential sensitivity to pinometostat therapy (2/2 CR pts had t(11;19)). Analysis of the baseline PBMC RNASeq data revealed 201 genes as significantly correlated with TOS (FDR adjusted P<0.05), these data were further analyzed using the Selventa causal modelling platform to identify pathways associated with TOS. Increased activity in pathways leading to differentiation, oxidative stress, inflammation, ras activation and decreased activities of PPAR-γ, DNA methylation and stem cell renewal were associated with increased TOS. Independent analysis of the cell line response data identified pathways that significantly overlapped with those observed in pt samples, with 4/7 of the pt derived mechanisms identified (PPAR-γ activity, inflammation, stem cell renewal and ras activation). In all cases directionality of pathway activity relative to pinometostat sensitivity were concordant between pt and cell line data. Based on these results the effect of a PPAR-γ agonist e.g. rosiglitazone, would be predicted to be antagonistic to pinometostat activity. Results of experiments investigating the effects of combination of rosiglitazone and pinometostat in MOLM-13 cell lines demonstrated that the combination was significantly less effective at inhibiting cell proliferation than pinometostat alone (IC50 shift of 2 fold (n=2)).

MLL-r fusion partner (e.g. t(11;19)) may influence clinical response to pinometostat. In addition RNASeq based characterization of patient samples and cell lines revealed candidate pathways that may cooperate with or antagonize pinometostat activity that warrant further investigation.
C13 Direct binding of ER\textalpha{} to YB-1 suppresses HER2 expression in human breast cancer. Tomohiro Shibata\textsuperscript{1}, Kosuke Watari\textsuperscript{1}, Hiroti Izumi\textsuperscript{2}, Akihiko Kawahara\textsuperscript{3}, Satoshi Hattori\textsuperscript{7}, Chihiro Fukumitsu\textsuperscript{4}, Yuichi Murakami\textsuperscript{5}, Ryuji Takahashi\textsuperscript{6}, Uhi Toh\textsuperscript{7}, Ken-ichi Ito\textsuperscript{8}, Maki Tanaka\textsuperscript{9}, Masayoshi Kage\textsuperscript{5}, Michihiko Kuwano\textsuperscript{5}, Mayumi Ono\textsuperscript{1}. \textsuperscript{1}Kyushu University, Fukuoka, Japan; \textsuperscript{2}University of Occupational and Environmental Health, Kitakyushu, Japan; \textsuperscript{3}Kurume University Hospital, Kurume, Japan; \textsuperscript{4}Kurume University, Kurume, Japan; \textsuperscript{5}St. Mary's Institute of Health Sciences, Kurume, Japan; \textsuperscript{6}Kyushu Medical Center, Fukuoka, Japan; \textsuperscript{7}Kurume University School of Medicine, Kurume, Japan; \textsuperscript{8}Shinshu University School of Medicine, Matsumoto, Japan; \textsuperscript{9}Kurume General Hospital, Kurume, Japan.

Background: ER\textalpha{} and HER2 are two major biomarkers of therapeutic efficacy in breast cancer patients. Y-box binding protein YB-1 is an oncprotein involved in breast cancer and a transcription factor for HER2/ErbB2. However, regulatory mechanisms of ER\textalpha{} and HER2 expression by YB-1 are largely unknown. In our present study, we examined how nuclear YB-1 regulates expression of HER2 and ER\textalpha{} in breast cancer cells.

Materials and methods: We established YB-1/Tet-On system in which nuclear YB-1 expression was induced by doxycycline, and examined how YB-1 could modulate expression of HER2 and ER\textalpha{} in breast cancer cells. Furthermore, we established the ER\textalpha{}/Tet-On system, in which ER\textalpha{} expression was markedly induced after treatment with doxycycline. To assess whether the YB-1-HER2-ER\textalpha{} correlation was affected by menopause, we analyzed biopsy samples of 116 premenopausal and 114 postmenopausal patients, who had not received any therapeutic drugs, by IHC.

Results: We first examined whether YB-1 regulates expression of ER\textalpha{} and HER2 by using various human breast cancer cell lines, and observed following findings. (1) Nuclear YB-1 overexpression increased HER2 expression and decreases ER\textalpha{} expression, while HER2 expression was suppressed by estradiol and enhanced by anti-estrogen drugs. (2) ER\textalpha{} binding to YB-1 suppressed YB-1 binding to the HER2 promoter region. Furthermore, binding of YB-1 to ER\textalpha{} was enhanced by estradiol and suppressed by anti-estrogen drugs. (3) YB-1 induced proteasomal degradation of ER\textalpha{} when both interact directly.

We have next examined whether expression of HER2, nuclear YB-1, and ER\textalpha{} are significantly associated by IHC analysis of biopsy samples of breast cancers. In breast cancers of postmenopausal, but not premenopausal patients, nuclear YB-1 expression was positively correlated with HER2 expression and negatively correlated with ER\textalpha{} expression.

Conclusions: In our study, we presented a new finding that YB-1 promoted proteasomal degradation of ER\textalpha{} by direct interaction and that YB-1-induced HER2 expression was suppressed by ER\textalpha{}. Furthermore, in breast tumors of postmenopausal patients, nuclear YB-1 expression was positively correlated with HER2 expression and negatively correlated with ER\textalpha{} expression. Therefore, this study could contribute to further development of optimized endocrine- and HER2- targeted therapeutics against breast cancer.


Background: The breast cancer type 1 susceptibility protein (BRCA1) carries out a primary function in the DNA homologous recombination repair (HRR) pathway and mutations in the BRCA1 gene have been linked
to a dramatic incidence in breast and ovarian cancer. BRCA1 associated cancers also lack HRR and are reliant on other DNA repair pathways such as base excision repair (BER) and non-homologous end joining (NHEJ). Three primary proteins involved in BER and NHEJ are Poly(ADP-ribose) Polymerases (PARP) 1, 2 and 3, and inhibition of these proteins lead to a synthetic lethality in BRCA1 mutated tumors. Despite unveiling structural and mechanistic properties of BRCA1 and the PARP enzyme super family, PARP inhibition is only effective in relatively low percentages of patients who possess BRCA1 mutations. Therefore, the need for biomarkers to predict patient response to PARP inhibition is highly important. Our lab has developed a radio-iodinated-PARP inhibitor (iodine-125-KX1) capable of quantitative measurements of active PARP enzymes in whole cell assays that can predict the in vitro response to PARP inhibitors.

Methods: [125I]KX1 was synthesized through radio-iododestannylation and purified by high-performance liquid chromatography. BRCA1 mutated and non-mutated ovarian and breast cancer cell lines: SNU-251, SKOV3, HCC1937, and MDA-MB-231 were investigated in a live cellular assay with [125I]KX1. Saturation, competitive inhibition, and kinetic assays were performed in all cell lines using [125I]KX1. Western analysis was performed to measure baseline PARP1, Poly(ADP-ribose(PAR), and BRCA1 protein expression. PARP inhibitor efficacy of talazoparib and olaparib was assessed through modified clonogenic assays. [125I]KX1 biodistribution experiments were carried out in mice bearing HCC1937 and MDA-MB-231 tumors to examine PARP in vivo in BRCA1 mutated and non-mutated cancer cell lines.

Results: [125I]KX1 was synthesized in high radiochemical purity. Saturation experiments revealed that BRCA1 mutated cancer cell lines had a higher PARP binding potential measured by [125I]KX1. Subtle differences in PARP inhibitor affinity was noticed in the different cell lines through competitive inhibition assays. Kinetic analysis revealed that the SNU-251 had the slowest dissociation kinetics of [125I]KX1. Western analysis confirmed PARP activity measured immunohistochemically by PAR correlated with PARP binding potential measured with [125I]KX1. In vitro PARP inhibitor potency was strongly correlated with PARP binding potential. Biodistribution studies revealed that PARP can be measured quantitatively in vivo.

Conclusion: With the utilization of [125I]KX1 we have been able to explore the PARP enzyme family in relation to BRCA1 mutations using in vitro and in vivo breast and ovarian cancer models. Quantitative measurements of PARP enzymes in live cancer cells has not been reported, and provides new insight into understanding the molecular target for PARP inhibitor therapy. Our data shows there is a positive correlation with PARP enzyme binding potential and response to PARP inhibitor therapy in vitro. Differences in PARP binding potential can also be measured in vivo and offer a prognostic biomarker marker for patients who may receive PARP inhibitor therapy.

CI6 The use of an antibody independent method, ApoStream™, to isolate circulating tumor cells (CTCs) isolated from non-small cell lung cancer patients and identification of EGFR mutations. Hai T. Tran1, Tsao S. Anne1, Katherine Richardson2, Ben Legendre2, Asifa Haider2, Darren Davis2, John Heymach1. 1UT MD Anderson Cancer Center, Houston, TX; 2Transgenomic, Inc, Omaha, NE; 3Apocell, Inc, Houston, TX.

Background: A variety of methods for capture of rare CTCs of epithelial origin are available; most employ antibodies to epithelial cell adhesion molecule (EpCAM) and cytokeratin (CK). Using a classic phenotypic definition, a CTC is a nucleated, CK (+), CD45(-) cell. However, some CTCs may elude capture as they originate from primary tumor cells that have undergone epithelial-mesenchymal transition (EMT). We report here the use of ApoStream™, a novel dielectrophoresis field-flow-assisted, antibody-free method to isolate CTCs from blood.
Methods: Blood was collected from consented NSCLC patients and processed using ApoStream™. For CTC enumeration comparison, the CellSearch® FDA-approved kit was used. Isolated cells were evaluated with a multiplexed immunofluorescent assay and laser scanning cytometry was applied to identify multiple combinations of positive and/or negative staining for CK/CD45/DAPI and EpCAM. To determine specific EGFR mutations from captured CTCs, samples were analyzed using Improved and Complete Enrichment with CO-amplification at Lower Denaturation temperature (ICE COLD-PCR).

Results: Blood samples from 40 NSCLC patients and 12 healthy volunteers were processed. In the normal, healthy volunteers, ApoStream™ isolated 0-1 CK(+)/CD45(-) cells and 0-33 CK(+)/CD45(+) cells. From the 38 of 40 NSCLC patients, ApoStream™ identified 0 to 65 CK(+)/CD45(-) CTCs, 2 samples failed in processing. Additionally, ApoStream™ recovered 37-3536 CK(-)/CD45(-) and 4-10702 CK(+)/CD45(+) cells. EpCAM expression was detected in 7-100% of CK(+)/CD45(-) and 0-5% of CK(-)/CD45(-) cells, and 18-100% of CK(+)/CD45(+) cells. In comparison, CellSearch® isolated 0 to 13 EpCAM(+)/CK(+)/CD45(-) CTCs in 7 patient samples tested. From our whole-blood spiked cancer cell (H1600, H1975) experiments, CTC recovery ranged from 13% to 60% with detection of EGFR mutations in as low as 10 recovered cells by ICE COLD PCR. From the 35 patients where CTCs were isolated by ApoStream, ICE COLD PCR correctly identified mutation status in 12 cases with EGFR exon 19 deletions (5), exon 21 - L858R(2) and wild type in 5 cases. There were 6 cases with either exon 18 or 20 mutation from tissue analysis that tested negative for exon 19 and 21 by ICE COLD PCR; exon 18 and 20 were not tested at this time. Mutation status was not detected in 16 cases when compared to tumor tissue analysis by Sanger sequencing. In 1 case, tissue revealed exon 19 - 15bp deletion meanwhile, CTC-ICP detected mutation at exon 21. Mutations were not tested in 5 CTC samples.

Conclusions: The ApoStream™ platform enriched EpCAM(+) and EpCAM(-) CTCs from the blood of NSCLC patients demonstrating utility in recovering cancer cells with multiple phenotypes. From a subset of samples, higher number of CK(+)/CD45(-) cells were recovered by ApoStream™ than CellSearch®. Furthermore, from recovered CTCs, detection of EGFR mutations was possible indicating the clinical relevance and utility of CTCs as an alternative to tissue biopsy.

C17 Role of miR-135b in gemcitabine sensitivity for metastatic breast cancer patients. Anna Tessari1, Dario Palmieri1, Giovanni Nigita1, Dario Veneziano1, Sara Cresta2, Biagio Paolini2, Maria Silvia Cona2, Taylor Vargo1, Erika Reese1, Tyler Sheetz1, Vincenzo Coppola1, Filippo De Braud2, Carlo M. Croce1. 1The Ohio State University, Columbus, OH; 2Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy.

Background: MicroRNAs are a class of small non-coding RNAs that modulate gene expression at post-transcriptional level, resulting crucial in many physiological and pathological processes. Their involvement in cancer initiation and tumor progression has been clearly established, as well as their linkage with chemoresistance. Gemcitabine is a nucleoside analog used in the treatment of metastatic breast cancer (MBC), characterized by a favorable safety profile. Only a small percentage of pts show a strong and prolonged response to this drug, thus suggesting the need of predictive biomarkers. Here, we aimed to identify microRNAs whose expression could be related to gemcitabine sensitivity in MBC.

Methods: 24 MBC pts were treated with gemcitabine as single-agent therapy from 1999 to 2011 at Fondazione IRCCS Istituto Nazionale Tumori (Milan). They were selected and classified by the response to the treatment (11 gemcitabine sensitive, stable or partial response after >24 weeks of treatment, and 13 gemcitabine resistant, progressive disease within 16 weeks from the first drug administration). MicroRNA expression profiling was performed by Nanostring nCounter Technology using RNA from FFPE metastatic lesions. Biological normalization was executed to correct for differences in sample abundances. Each sample
was normalized to the geometric mean of the top 100 most highly expressed microRNAs. Student’s t test was used to calculate statistical significances of pairwise comparisons. The nCounter analysis was validated on a wider population of 50 MBC pts (17 sensitive and 33 resistant) by qRT-PCR.

The correlation between microRNA expression levels and gemcitabine half maximal inhibitory concentration (IC50) was evaluated using different BC cell lines. Finally, gemcitabine sensitivity was assessed on these cell lines by cell survival assays following exogenous modulation of microRNA expression levels.

Results: Median age at diagnosis was 47 yrs (range 21-74 yrs). Hormone receptors were positive in thirty-one pts (62%), while Her2 in 15 cases (30%). Soft tissues were the most frequent site of metastasis (84%), followed by bone (64%), liver (36%), lung (34%) and CNS (16%). Sixteen pts (32%) had local recurrences. Median number of chemotherapies and endocrine therapies received prior to gemcitabine was 3 (range 1-6) and 2 (range 1-5) respectively.

From the nCounter analysis, a subset of 20 microRNAs was significantly deregulated (p<0.001) between sensitive and resistant pts. Among them, the three most relevant (miR-135b, miR-146b and miR-155) were selected for further investigation. RT-PCR validation confirmed microRNA expression profiling data on the wider cohort of 50 pts (p<0.05). In vitro studies confirmed the correlation between miR-135b levels and gemcitabine sensitivity in different BC cell lines. Moreover, the exogenous upregulation of this microRNA increased the response to the drug.

Conclusions: We identified miR-135b, miR-146b and miR-155 as potentially predictive for gemcitabine sensitivity in MBC pts. In particular, miR-135b appears to be causally involved in BC cells response to gemcitabine. These results pave the way to a better understanding of the molecular mechanisms underlying gemcitabine resistance in BC, and may have a clinical impact to identify those patients expected to obtain strong and prolonged benefits from this well-tolerated treatment.

C18 Epithelial and mesenchymal phenotypic characterization and mutation detection in circulating tumor cells isolated from peripheral blood of non-small cell lung cancer patients with ApoStream® technology. Priya Balasubramanian¹, Vishal Gupta¹, David Hasegawa¹, Carmen Y. Lam¹, Ran An¹, Swetha Pratyusha Gunteru¹, Hai T. Tran², John V. Heymach², Darren W. Davis¹. ¹ApoCell, Inc., Houston, TX; ²The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: The presence of circulating tumor cells (CTCs) has been established as a prognostic indicator in a few metastatic cancers. Although technically challenging, isolation and characterization of CTCs at the protein or nucleic acid level has significant potential to enable the development of minimally invasive methods to detect molecular alterations for personalized cancer targeted therapies. The goal of this study was to demonstrate the ability to enrich phenotypically heterogeneous population of CTCs and detect EGFR and KRAS mutations in isolated CTCs obtained from peripheral blood of patients with Non-small cell lung cancer (NSCLC) using ApoStream®, an antibody-independent CTC capture device based on dielectrophoresis technology.

Methods: For condition optimization, blood samples from a small cohort of cancer patients (n=8) were collected under an IRB approved protocol and processed with multiple ApoStream® operating frequencies (45, 55, 65 and 85 kHz). To evaluate clinical feasibility, blood samples (8 mL) from 35 additional NSCLC patients (with or without targetable “driver” mutations) and 10 normal, healthy donor controls were collected and processed using the optimized ApoStream® conditions. CTC enumeration was performed using a multiplex phenotyping panel targeting hematopoietic (CD45/CD15), epithelial (EpCAM/CK/E-
Cadherin) and mesenchymal (Vimentin/N-cadherin/Twist-1) markers. In addition, mutation detection assays were validated for EGFR (E746_A750, L858R, and T790M) and KRAS (G12C, and G12S) with mutant DNA spiked into wild type DNA at multiple dilutions using the droplet-based RainDrop® digital PCR system.

Results: An ApoStream® operating frequency of 85 kHz resulted in higher CTC counts and hence was selected for processing samples for CTC enumeration assay and a lower operating frequency of 65 kHz was selected to provide higher purity for processing samples for mutation analysis. Preliminary data demonstrated detectable CTCs (above healthy control baseline of 4 CTCs per mL blood) in 7 out of 10 patient samples processed to date (mean=24; median =14 and range= 5-100 CTCs per mL blood). CTCs isolated from all 7 patients exhibited mesenchymal phenotype. In addition, two patients also showed CTCs of epithelial phenotype demonstrating that the ApoStream® platform enriches phenotypically heterogeneous CTCs. Sensitivity of mutation detection determined from dilution series was found to be assay dependent; limit of quantitation (LOQ) for EGFR and KRAS mutations were found to be 0.01% or higher while the linearity for all the assays demonstrated a linear regression coefficient (R2) greater than 0.95 except for EGFR L858R which requires further analysis. Preliminary data from 8 NSCLC patient samples demonstrated the ability to detect mutations in ApoStream® enriched CTCs. Enumeration and mutation analysis is in progress for the remaining patient samples.

Conclusion: We demonstrated the ability to isolate, enumerate and define epithelial and mesenchymal phenotypes of CTCs and to detect oncogenic mutations in CTCs isolated from NSCLC patient samples using ApoStream®.

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C19 Use of liquid biopsies in clinical oncology: The UCSD Moores Cancer Center experience in 168 patients with diverse malignancies. Maria C. Schwaederle, David E. Piccioni, Santosh Kesari, Hatim Husain, Sandip P. Patel, Razelle Kurzrock. Center for Personalized Cancer Therapy, UCSD Moores Cancer Center, La Jolla, CA.

There is a growing interest in using methods such as liquid biopsies to assess genomics of circulating tumor DNA (ctDNA) in patients with advanced cancer. Liquid biopsies are non-invasive, may provide access to shed DNA from multiple metastases, and the dynamic results of genomic evolution can be followed with repeat blood sampling.

We report our initial experience using ctDNA testing in 168 consecutive patients with diverse cancers. Testing was performed using a panel of 54 cancer-related genes (digital sequencing), examining mutations (in all 54 genes) as well as amplifications in ERBB2, EGFR, and MET (Guardant Health, Inc. (Guardant360 test)).

Fifty-eight percent of patients (98/168) had ≥ 1 alteration(s) identified in their plasma. In a multivariable analysis, gastrointestinal cancer (N=13) was an independent predictor of a higher number of alterations (median = 2 alterations/patient; P=0.001), while primary brain tumors (N=56) correlated with fewer alterations (median = 0 alterations/patient; P=0.019) detected in the plasma. Even so, 16 of 56 patients (28.6%) with primary brain tumors had discernible ctDNA alterations. Of the 98 patients with alterations, 71.4% had ≥ 1 anomaly potentially actionable by an FDA-approved drug. When comparing results of patients (N = 63) who had both a tissue and ctDNA test performed (and had aberrations in the tissue biopsy that were detectable by the ctDNA panel), 22 individuals (35%) had ≥ 1 alteration in common between the tissue and ctDNA test (median time interval between the tissue biopsy and the blood draw = 2.7 months for these
22 patients). In contrast, 41 patients had no alterations in common between tissue and ctDNA, and in these 41 individuals, the median time between tissue biopsy and blood draw was 14.4 months (P=0.006). Overall concordance rates were 70.3% for TP53 and EGFR, 88.1% for PIK3CA, and 93.1% for ERBB2 alterations. In addition, we observed a strong correlation between cases with $\geq 1$ alteration reported with ctDNA $\geq 5\%$ and shorter overall survival (median = 4.0 months versus not reached; P<0.001 in multivariable analysis; median follow up time = 6.1 months). Finally, 5 of 12 patients (42%) matched to a therapy according to their ctDNA test results achieved stable disease $\geq 6$ months (N = 2) or partial response (N = 3).

Taken together, ctDNA testing via liquid (blood) biopsies demonstrated frequent potentially actionable aberrations, including in primary brain tumors. The longer the time elapsed between tissue and ctDNA procurement for testing, the less chance that genomic alterations would be consistent between the two specimens. Higher percentage of ctDNA was an independent predictor for shorter survival. Finally, preliminary results suggest that patients matched to cognate targeted treatments based on ctDNA can achieve clinical benefit. Overall, these observations indicate that ctDNA genomic analysis may be a valuable tool for evaluating patients with cancer.

Cancer Stem Cells

C20  Reversal of mesenchymal phenotype of triple-negative breast cancer and glioblastoma cells and its therapeutic implication. Kiyotsugu Yoshikawa1, Hiroaki Sakai1, Masahiro Shimazaki1, Nobuhiro Okada1, Yoshiaki Matsumoto1, Ellis Reinherz2, Masakazu Toi1. 1Kyoto University Graduate School of Medicine, Kyoto, Japan; 2Dana-Farber Cancer Institute, Boston, MA.

Mesenchymal transition is common to many aggressive cancers including breast, glioblastoma (GBM), lung and pancreatic cancers characterized by chemo/radiotherapy-resistance and cancer stem cell-like property. Claudin-low mesenchymal subtype in triple-negative breast cancer (TNBC) is a major problem due to the lack of molecularly-targeting drugs. So far, we have identified an shRNA (shP1) which can induce mesenchymal to epithelial transition (MET) in shRNA library screening using positive selection reporter system in MDA-MB-231 mesenchymal TNBC cell line. Here, we have analyzed the effect of shP1 on morphology, transcription and cell proliferation in various mesenchymal cancer cell lines derived from breast, glioblastoma (GBM), lung and pancreatic cancers.

The shP1 increased E-cadherin promoter-driven GFP in a wide range of mesenchymal cancers including brain, breast, pancreatic, lung origin. Drastic morphology change introduced by shP1 was observed both in 2D and 3D cultures (matrigel, collagen), reducing their invasive and branching morphogenesis, especially in U251 GBM and MDA-MB-231 TNBC cell lines. The shP1-introduced cells are more sensitive to EGFR-inhibition and paclitaxel in TNBC cell line, reminiscent of reversal of therapy-resistant phenotype. Gene expression analysis reveals alteration of pathways related to cell adhesion, ERBB signal pathway, apoptosis and cytokines signaling. Of note, shP1-introduced U251 cells increased gene expression characteristic of proneural subtype, indicating shP1 reverted the subtype from mesenchymal to proneural. These results suggest that although U251 and MDA-MB-231 cell lines are of different tissue origins, their mesenchymal property can be reverted by the same program targeted by single shRNA.

These results provide a rationale of MET as therapeutic for a wide range of cancers with mesenchymal property.
C21  Generation of Wnt- and mitogenic receptor binding bispecific antibodies to target cancer stem cells. Berina Eppink1, Rob Roovers1, Bram Herpers2, Wim de Lau3, Carina Clements4, Vanessa Zondag van der Zande5, Abdul Basmeleh1, Willem Bartelink1, Marc van de Wetering4, Robert Vries4, Leo Price2, John De Kruif6, Mark Throsby1. 1Merus BV, Utrecht, Netherlands; 2OcellO BV, Leiden, Netherlands; 3Hubrecht Institute, Utrecht, Netherlands; 4Hubrecht Institute/Hubrecht Organoid Technology (HUB), Utrecht, Netherlands.

Background: In colorectal cancer (CRC) and other solid tumors, cancer stem cells (CSC) contribute to tumor progression and resistance to standard chemotherapies. The continuous regeneration of the colon is dependent on strict control of developmental (e.g. Wnt) and mitogenic (e.g. EGF) pathway signaling; dysregulation results in uncontrolled proliferation forming the basis of aggressive tumors with metastatic potential. Here we describe the generation of novel bispecific antibodies designed to target CSC through Wnt signaling receptors and block growth factor signaling. The Wnt targets LGR5, LGR4, ZNRF3 and RNF43 were selected since their expression is modulated in CSC populations. The GPCR family members LGR4/LGR5 are positive Wnt regulators and the transmembrane E3 ligases ZNRF3/RNF43 are negative Wnt regulators. The growth factor receptor EGFR is frequently (>70%) overexpressed in CRC and its blockade has demonstrated clinical benefit in a subgroup of patients. More recently, HER3 pathway activation has been implicated in resistance to EGFR-targeted therapies.

Experimental procedures and results: Two parallel strategies were applied to generate panels of common light chain (cLC) Fab against LGR4, LGR5, ZNRF3 and RNF43. Humanized cLC mice (MeMo®) were immunized with recombinant protein or DNA, and materials harvested from these mice used to generate Fab regions against these antigens. The second approach utilized large and diverse synthetic cLC Fab-phagemid libraries. Combined, these methods resulted in ~1500 unique antigen-specific Fab from which ~300 were selected for further testing.

Bispecific antibodies were produced in a human cLC IgG1 format using substitutions in the IgG Fc regions for coexpression of two different heavy chains resulting in the generation of large panels of pure and stable bispecific IgG suitable for screening. The Wnt target specific Fab were combined with a Tetanus toxoid-specific control Fab arm allowing for stringent ranking of these Wnt-specific panels in a monovalent format for specificity, affinity, stability, and ligand (R-Spondin3) blocking potency. Based on this characterization the 54 most promising Wnt targeting arms were combined with a panel of previously characterized EGFR and HER-3 specific Fab arms resulting in ~ 500 different cLC bispecific IgG for functional testing. All bispecific IgG were screened for potency of growth inhibition of CSC using novel 3D high content imaging readouts on patient-derived CRC organoids. The organoids are cultured using growth factors that allow for the maintenance and proliferation of healthy and diseased stem cells and their offspring. Functional analysis revealed several bispecific antibodies that inhibited CRC organoid growth much more potently than comparator drugs such as cetuximab or erlotinib.

Conclusion: Bispecific antibodies present a biological modality that result in unexpected functional activities by mechanisms possible unique to the architecture of these molecules. Identifying these unique properties requires the rapid generation and screening of large panels of bispecific IgG directly in the therapeutic format in relevant functional assays. Initial screening results of the bispecific antibodies targeting Wnt and HER family members supports the concept of CSC targeting and several leads are currently undergoing more extensive characterization.
A phase I/II study of the CXCR1/2 inhibitor Reparixin in combination with weekly paclitaxel in metastatic HER2 negative breast cancer - final analysis. Anne F. Schott1, Max S. Whicha1, Raymond P. Perez2, Giraldo Kato3, Tiffany Avery4, Massimo Cristofanilli5, James M. Reuben6, R. Katherine Alpaugh6, Pier Adelchi Ruffini7, Susan Mccanna7, Lori J. Goldstein6. 1Comprehensive Cancer Center University of Michigan, Ann Arbor, MI; 2University of Kansas Medical Research Center, Fairway, KS; 3Oncology Research Associates, Pinnacle Oncology Hematology, Scottsdale, AZ; 4Thomas Jefferson University, Philadelphia, PA; 5Department of Hemopathology research, MD Anderson Cancer Center, Houston, TX; 6Department of Medical Oncology, The Hospital of Fox Chase Cancer Center, Philadelphia, PA; 7Dompé farmaceutici s.p.a., Milano, Italy.

Background. Cancer Stem Cells (CSC) have the ability to self-renew and generate the full range of cells that make up a bulk tumor. Experimental models and retrospective clinical observations point to CSC as responsible for tumor recurrence and metastasis. An ideal CSC targeting agent should be a non toxic molecule that can be safely administered also in combination with chemotherapy to improve disease control. CXCR1, one of the receptors for CXCL8, has been identified on breast cancer CSC. Reparixin, an allosteric inhibitor of CXCR1, reduced CSC in breast cancer (BC) xenografts (Ginestier C et al., JCI 2010) both as single agent and in combination with taxane chemotherapy.

Methods. Patients were female aged ≥ 18 years with HER-2 negative metastatic BC, non taxane-refractory, who had received up to 3 prior chemotherapy (CT) lines for advanced BC (not including neo/adjuvant CT), had measurable disease according to RECIST 1.1, ECOG PS of 0-1, adequate organ function, and no brain metastases. Patients received a 3-day run-in with reparixin oral tablets 3 times daily (tid) followed by paclitaxel 80 mg/m² (days 1, 8, and 15 for 28-day cycle) + reparixin oral tablets tid for 21 days. Three dose levels of 3-6 subjects were explored: 400 mg, 800 mg and 1200 mg oral reparixin tid. The highest safe dose level was expanded twice to gain additional safety and activity data. Treatment continued until disease progression, unacceptable toxicity or withdrawal of consent. Primary endpoints were safety and tolerability, and pharmacokinetic (PK) profile of the combination treatment. Among secondary endpoints, assessment of disease response every 2 cycles for indication of efficacy and correlative evaluations on peripheral blood samples were conducted. First analysis (i.e., 60 days post last patient in, LPI) of the results from this trial was reported earlier (Schott AF et al., SABC 2014).

Results. Herein we report data at 6 months post LPI. From 02/2012 to 04/2014, 33 patients entered the study (4 in cohort 1, 3 in cohort 2 and 26 in cohort 3). 30 patients were evaluable for safety. Neither grade 4 adverse events (AE) nor Serious AE related to reparixin were reported. 9/23 patients at the highest dose level reported Grade 3 AE among which granulocytopenia (3 patients) and peripheral neuropathy (2 patients) that are commonly seen with paclitaxel alone. Overall, 8 confirmed responses (2 CR, 6 PR) were observed among 26 patients who underwent at least 1 tumor assessment (every 8 weeks). Response duration (days) was 645+, 466+ (for CR) and 280+, 169, 141, 113+, 47 (for PR). Two additional patients experienced SD > 6 months (318 and 288 days, respectively). Of responding patients, all but one was from cohort 3. Median TTP (days) in the safety population was 58, 67 and 170 in cohorts 1, 2 and 3, respectively.

Conclusions. Combination treatment demonstrated good tolerability with low incidence and severity of adverse reactions. The recommended dose of reparixin for the combination was established at 1200 mg tid. A sizeable response rate and mTTP was recorded, with some interesting long term responders. A randomized phase II study of the combination versus single agent weekly paclitaxel in frontline treatment of patients with metastatic triple-negative BC is ongoing (NCT02370238).
C24 Functional phenotypic screening of bi-specific antibodies in genetically heterogeneous colon carcinoma organoids. Bram Herpers¹, Rob Roovers², Berina Eppink², Marc van de Wetering³, Kuan Yan¹, Lucia Salinaro¹, Wim de Lau³, Hans Clevers⁵, Robert Vries⁵, Mark Throsby², Leo S. Price³. ¹OcellO B.V., Leiden, Netherlands; ²Merus B.V., Utrecht, Netherlands; ³Hubrecht Institute, Utrecht, Netherlands.

Background. The relationship between cell/tissue morphology and disease state forms the basis of modern histopathology. In an in vitro experimental setting, cell and tissue morphology can be used to identify potential drugs, discriminate different modes of drug action and discriminate between therapeutic activity and toxicity. The high failure rate in cancer drug research has been linked to the poor predictive capacity of 2D in vitro culture models. Compared to 2D monolayer cultures, 3D cultured tissues show gene expression, differentiation and functional characteristics which more closely reflect the situation in vivo. Furthermore, complex 3D tissue architecture exemplified by organoid cultures increases the scope for discriminating different drug responses. In this study we used 3D image analysis of a panel of 20 sequenced colorectal cancer organoids to characterize the genotype-phenotype relationship and the response to a broad spectrum of inhibitors of signaling pathways. This high throughput approach was then used as a primary screen to evaluate the functional activity of a panel of 550 bispecific antibodies comprised of a HER3 or EGFR targeting arm combined with a LGR4, LGR5, ZNRF3 or RNF43 targeting arm to target stem cells.

Results. Diverse morphological features were extracted from 3D image data, including organoid size and shape, planar cell polarity, lumen formation as well as cell number and nucleus shape. The broad heterogeneity of mutation spectra of the organoids was reflected in broad heterogeneity of organoid phenotypes. Some CRC organoids formed well differentiated spheroids with a single lumen that resembled the phenotype of normal wild type organoids, whereas others had multiple lumens or were poorly differentiated without a luminal cavity. Some features, such as extent of lumen formation, were more sensitive to drug treatment than cell proliferation-associated features (e.g. organoid size or number of cells per organoid). This improved the sensitivity of the assay to detect active molecules. A set of 10 features was selected to create a drug response profile. We observed that the presence of activating mutations did not always correlate with sensitivity to corresponding pathway inhibitors, underscoring the need for empirical testing of drugs to predict patient sensitivity. To reflect key mutational subtypes, from the panel of 20 CRC organoids, APC mutant, APC mutant/SMAD4 mutant and APC wildtype/RNF43 mutant organoids were selected to screen the bispecific antibody panel. Several bispecific antibodies were identified that potently inhibited growth of the CRC organoids with differing sensitivities dependent on mutational background.

Conclusions. These results demonstrate that high content screening of CRC organoids is an effective strategy to identify novel inhibitors of CRC tumor growth and enable identification of bispecific antibodies that target colorectal cancer stem cells with different mutational backgrounds.


We hypothesise that cancer stem cells with high aldehyde dehydrogenase (ALDH<sup>high</sup>) activity present a new therapeutic target and will be selectively sensitive to 5-nitrofuran pro-drugs.

Cancers are heterogeneous and contain subpopulations of ALDH<sup>high</sup> cells with tumour initiating potential. ALDH enzymes metabolize toxic aldehydes, and are highly expressed in somatic and cancer stem cells (CSCs), although their function in stem cells is not fully understood. In a small molecule screen coupled with target ID, we recently discovered that clinically active 5-nitrofurans (5-NFNs) are substrates of ALDH2
(Zhou et al., 2012). 5-NFNs are a class of pro-drug widely used to treat bacterial and parasitic infections where their relative specificity is driven by nitroreductases, but little is known about the enzymes that bio-activate 5-NFNs in humans. Recent clinical cancer research has found that the 5-NFN nifurtimox has anti-cancer properties and it is currently in Phase 2 clinical trials for neuroblastoma and medulloblastoma (ClinicalTrials.gov Identifier: NCT00601003), however the mechanism underlying this anti-cancer activity is unknown.

In melanoma and other cancers, ALDH1A1 and ALDH1A3 are highly expressed in CSCs. We find that cancer cell lines are highly sensitive to 5-NFNs in cell viability assays, where we use a logarithmic drug dose range and assess cell viability by PrestoBlue™ (e.g. A375 melanoma cells EC$_{50}$=86nM). To test if ALDH1 isoforms are substrates of 5-NFNs, we preformed in vitro activity assays by monitoring NADH production ($\lambda$=340nm). We find that the clinically active 5-NFNs nifuroxazide and nifurtimox, in addition to our own newly synthesised 5-NFNs, are competitive substrates for human ALDH1A3 activity in vitro ($p<0.05$). Notably, nifuroxazide was not a substrate for ALDH2, suggesting that nifuroxazide may show selectivity toward ALDH1 isoforms. Consistent with our enzymatic activity assays, we find that 5-NFNs are competitive substrates for ALDH activity in melanoma cells by Aldefluor™ in vivo, with 5-NFNs displaying a prolonged competitive inhibition compared with the known inhibitor, DEAB. Importantly, no-nitro control compounds show no activity toward ALDH enzymes in vitro or in vivo. Computational docking studies reveal that 5-NFNs have the potential to fit within the interior of the ALDH enzymatic cavity and interact with the catalytic cysteine, thereby offering a potential mechanism for 5-NFN bio-activation. Kinetic living-cell imaging (IncuCyte ZOOM™) reveals that ALDH1A3 siRNA transfected A375 cells are protected from 5-NFN toxicity ($p>0.05$) and apoptosis ($DRAQ7$™: $p<0.0001$), demonstrating a functional role for ALDH1A3 in mediating 5-NFN activity in cancer cells.

Our work demonstrates a novel and biologically relevant 5-NFN-ALDH1 interaction in cancer cells. We propose 5-NFNs have the potential to target ALDH$^{\text{high}}$ CSCs within a tumour and advance the repurposing of clinical 5-NFN pro-drug antibiotics as anti-cancer therapeutics.

C26 Development of selective MELK kinase inhibitors for breast cancer treatment. Piotr Kowalczyk1, Paulina Węgrzyn1, Monika Prokopowicz1, Martyna Knop1, Karolina Mazur1, Katarzyna Dziedzic1, Karolina Gluza1, Martyna Knop1, Katarzyna Dziedzic1, Karolina Mazur1, Adam Radzimierski1, Claude Commandeur1, Magdalena Jawadzka1, Kristjan Bloudoff2, Fred Vaillancourt2, Nick Larsen2, John Wang2, Dom Reynolds2, Daisuke Ito3, Jian Zou2, Michelle Aicher2, Pete Smith2, Ping Zhu2, Krzysztof Brzózka1. 1Selvita SA, Kraków, Poland; 2H3 Biomedicine, Cambridge, MA; 3Eisai, Tokyo, Japan.

Breast cancer is the second most common cancer in the world and the most frequent cancer among women. Despite the progress in developing breast cancer therapies, approximately, 15% of all breast cancers are diagnosed as triple negative breast cancer (TNBC) and due to the lack of estrogen and progesterone receptors this subgroup of patients remains difficult to treat with hormonal therapies. Additionally, therapies targeting HER2, such as Herceptin, are also inefficient against TNBC. In recent years, maternal embryonic leucine zipper kinase (MELK) has been identified as a novel oncogenic target that is highly expressed in several types of solid cancers: breast (especially triple negative breast cancer), colon, ovary, lung, and brain and present at low levels in normal tissues. MELK overexpression in patient tumors strongly correlates with poor prognosis in glioblastoma and breast cancer. siRNA mediated knockdown of MELK kinase significantly inhibits growth of tumor cell lines both in vitro and in vivo. Therefore, MELK kinase is emerging as a novel and interesting target with significant potential for therapeutic intervention in cancer.
MELK is an atypical member of the AMPK family of serine-threonine kinases that have been implicated in stem cell renewal, cell cycle progression, cytokinesis, mRNA splicing, and apoptosis. Its activity is correlated with its phosphorylation level, is cell cycle dependent, and maximal during mitosis although direct upstream regulators of MELK kinase activity are unknown. Despite the fact that the exact function is currently under investigation, selective targeting of MELK may be an effective cancer treatment strategy in a wide range of solid tumors. In this study, we are reporting development of a series of selective MELK kinase inhibitors. Synthesized compounds exert excellent selectivity and potency in MELK inhibition in a low nanomolar range. Therapeutic effect of the compounds was investigated in the panel of breast cancer cell lines with different genetic background as well as with different MELK kinase levels; it was shown that for some cell lines compounds induced cell death with nanomolar ED50 values. The compound's effect on the proliferation and in the colony formation assay was also investigated. Taken altogether, the presented data supports our rationale of using MELK kinase inhibitors as a novel approach for the cancer therapy.

C27 Activation of PKA induces a mesenchymal-to-epithelial transition and epigenetic reprogramming-mediated loss of tumor initiating ability, Diwakar R. Pattabiraman, Robert A. Weinberg. Whitehead Institute for Biomedical Research, Cambridge, MA.

Cancer stem cells (CSCs) have emerged in recent years as important targets for cancer therapy owing to their elevated resistance to conventional chemotherapy as well as to tumor-initiating ability that enables them to seed new tumors and thereby drive clinical relapse. The epithelial-to-mesenchymal transition (EMT) is a cell-biological program that confers mesenchymal traits on both normal and neoplastic epithelial cells. By activating EMT programs, carcinoma cells acquire malignancy-associated traits and additionally may become converted into CSC-like cells, as has been shown in many cases. This association between the EMT program and the CSC state has presented an attractive opportunity for drug development and agents that preferentially target more mesenchymal carcinoma cells rather than their epithelial counterparts are highly sought. An alternative therapeutic approach is to convert CSCs to their non-stem cell counterparts through the induction of a mesenchymal-to-epithelial transition (MET). Here we observe that increases in intracellular levels of the second messenger 3’-5’-cyclic adenosine monophosphate (cAMP) and the subsequent activation of protein kinase A (PKA) induces an MET in mesenchymal human mammary epithelial cells and their neoplastic derivatives. The MET-induced differentiation is accompanied by a loss of stem-like properties and tumor-initiating ability, rendering the cells more sensitive to treatment with chemotherapeutic drugs such as doxorubicin and paclitaxel. Upon activation PKA phosphorylates and activates PHF2, a histone H3K9 demethylase, which relieves H3K9me2/3-mediated repression of epithelial genes. Genome-wide occupancy studies of PHF2 reveal that it interacts with numerous genomic loci, relieving their methylation-mediated silencing, enabling the access of epithelial loci to transcription machinery and ultimately, acquisition of an epithelial state. This works presents an attractive strategy to induce an MET through the activation of a histone demethylase that induces widespread epigenomic reprogramming and acquisition of an epithelial state that is more amenable to chemotherapeutic treatment.
C28  The combination of Acetazolamide and Sulforophane targets the tumor initiating cells in human bronchial carcinoids. Narges Baluch1, Reza Bayat Mokhtari2, Tina Homayouni2, Pedram Akbari2, Fatemeh Heshmati2, Syed S Islam3, Sushil Kumar3, Karen Aitken2, Carrie Fitzpatrick2, Herman Yeger2. 1Queen's University, Kingston, ON, Canada; 2The Hospital for Sick Children, Toronto, ON, Canada; 3The University of Chicago, Chicago, IL, Canada.

Bronchial carcinoids (BC) are neuroendocrine tumors that present as typical (TC) and atypical (AC) variants, the latter being more aggressive. The AC form of BC possesses a higher degree of invasiveness and metastatic potential. It is important to understand how the BC tumor initiating cells (TIC) or cancer stem cells (CSC) in BC account for this phenotype. In order to functionally characterize the suspected BC stem cell fraction, BC cell lines (H727 and H720) were grown under a non-adhesive condition to form 3D spheroids favouring expansion of the TIC. The third generation of spheroids was assessed for clonogenicity, tumorigenicity (xenograft model), stem cell and hypoxia markers, drug resistance, karyotype and microarray analysis compared to parental cells. We previously showed that a pan-carbonic anhydrase inhibitor, acetazolamide (AZ) significantly potentiated the anti-tumor effects of sulforophane (SFN, a natural isothiocyanate with HDACi activity) on these lung carcinoid cell lines in vitro and subcutaneous xenografts. Here we developed an orthotopic lung model to study tumor progression and metastatic behaviour of the spheroid forming BC cells. Orthotopically grown tumors were treated with AZ, SFN and the AZ+SFN combination using previous doses within clinically available ranges. Results demonstrated that the spheroid grown BC cells showed increased clonogenic, tumorigenic, and drug resistance potential, with enhanced expression of stem cell, carbonic anhydrase and hypoxia markers as compared with the control non-spheroid cells, all suggesting selection for the TIC or CSC fraction. As in the subcutaneous model the combination of AZ with SFN was more effective than either single agent alone in inhibiting orthotopic tumor growth and survival. Taken together the evidence indicates that the novel AZ+SFN combination is likely targeting the TIC/CSC population. This offers increased value in terms of the management of BC tumor progression and possible metastasis in BC patients presenting with more aggressive variants.


Targeting cancer stem cells (CSCs) holds promise to address key challenges of cancer treatment: chemotherapy and radiation therapy resistance, metastasis, and recurrence. Increased CSC abundance after neoadjuvant chemotherapy has been associated with a significantly worse outcome. Combining CSC-targeted agents with chemotherapy may lead to more durable response with increased overall survival.

Focal adhesion kinase (FAK), a non-receptor tyrosine kinase, mediates signal transduction by integrins and growth factor receptors to regulate cellular adhesion, proliferation, migration, and survival. Several studies have demonstrated that FAK expression and kinase activity are necessary for survival and maintenance of CSCs. FAK is also upregulated in many epithelial tumors and associated with poor patient prognosis and therefore has been pursued as a promising therapeutic target for cancer. VS-6063 and VS-4718 are orally bioavailable small molecules that impede CSCs through the inhibition of FAK. Both VS-6063 and VS-4718 preferentially kill CSCs in multiple cancer models, including models of breast, ovarian, SCLC, and mesothelioma, and these agents are in clinical development. We demonstrate that treatment of cancer cell lines or mice bearing xenograft tumors with VS-6063 or VS-4718 decreases CSCs as assessed by decreased tumor-initiating capability in limiting dilution assays. Both FAK inhibitors decrease significantly CSCs to a varying degrees across multiple tumor models. Furthermore, treatment of mice with either FAK inhibitor following cessation of chemotherapy delayed tumor regrowth in xenograft and PDX models of TNBC, SCLC,
and mesothelioma. A mechanistic investigation in breast cancer cell lines revealed an important crosstalk between FAK and the Wnt/β-catenin pathway, where FAK inhibition blocked tyrosine-654 phosphorylation and β-catenin activation. Importantly, a constitutively active mutant form of β-catenin “rescued” CSCs, suggesting that preferential targeting of CSCs by FAK inhibitors is mediated, at least in part, through attenuation of downstream β-catenin activation.

In breast cancer, CSCs are identified by the expression of aldehyde dehydrogenase 1 (ALDH1) or CD44-high/CD24-low markers. To probe the CSC populations in breast cancer patients, we developed a multiplex immunofluorescence assay with three CSC markers (ALDH1, CD44, and CD24) and an epithelial marker (pan-cytokeratin) for FFPE tissue. Automated image analysis with pathology review was used to evaluate single CSCs within the tumor. This assay may provide a means of monitoring a neoadjuvant CSC-targeted treatment in breast cancer FFPE samples in clinical trials.

In summary, our results show that FAK inhibitors preferentially target cancer stem cells and delay the regrowth of tumor cells post standard-of-care treatment in mouse models providing rationale for the clinical development of FAK inhibitors to achieve more durable responses for cancer patients.

Cellular Responses to Therapy

C30 The 1,2-diaminocyclohexane carrier ligand in oxaliplatin induces p53-dependent transcriptional repression of factors involved in thymidylate biosynthesis. Hiroyuki Kitao1, Shinichi Kiyonari1, Makoto Iimori1, Kazuaki Matsuoka2, Tomomi Morikawa-Ichinose1, Daisuke Miura1, Shinichiro Niimi2, Hiroshi Saeki1, Eiji Oki1, Yoshihiko Maehara1. 1Kyushu University, Fukuoka, Japan; 2Taiho Pharmaceutical Co. Ltd, Tokushima, Japan.

Platinum-based chemotherapeutic drugs are widely used as components of combination chemotherapy in the treatment of cancer. One such drug, oxaliplatin, exerts a synergistic effect against advanced colorectal cancer in combination with 5-FU and leucovorin. In the p53-proficient colorectal cancer cell line HCT116, oxaliplatin represses the expression of deoxyuridine triphosphatase (dUTPase), a ubiquitous pyrophosphatase that catalyzes the hydrolysis of dUTP to dUMP and inhibits dUTP-mediated cytotoxicity. However, the underlying mechanism of this activity has not been completely elucidated, and it remains unclear whether factors other than downregulation of dUTPase contribute to the synergistic effect of 5-FU and oxaliplatin. In this study, we found that oxaliplatin and dachplatin, platinum-based drugs containing the 1,2-diaminocyclohexane (DACH) carrier ligand, repressed the expression of nuclear isoform of dUTPase (DUT-N), whereas cisplatin and carboplatin did not. Oxaliplatin induced early p53 accumulation, upregulation of primary miR-34a transcript expression, and subsequent downregulation of E2F3 and E2F1. Nutlin-3a, which activates p53 non-genotoxically, had similar effects. Introduction of miR-34a mimic also repressed E2F1 and DUT-N expression, indicating that this miRNA plays a causative role. In addition to DUT-N, oxaliplatin repressed, in a p53-dependent manner, the expression of genes encoding enzymes involved in thymidylate biosynthesis. Consequently, oxaliplatin significantly decreased the level of dTTP in the dNTP pool in a p53-dependent manner. These data indicate that the DACH carrier ligand in oxaliplatin triggers signaling via the p53-miR-34a-E2F axis, leading to transcriptional regulation that ultimately results in accumulation of dUTP and reduced dTTP biosynthesis, potentially enhancing 5-FU cytotoxicity.
Molecular Targets and Cancer Therapeutics

Poster Session C
Sunday, November 8, 2015 • 12:30 p.m.-3:30 p.m.

C31 Vemurafenib selectively radiosensitizes BRAF V600E mutant papillary and anaplastic thyroid carcinoma cells in vitro. Ryan N. Robb, Linlin Yang, Moumita Chatterjee, Moto Saji, Matt Ringel, Arnab Chakravarti, Terence Williams. The Ohio State University, Columbus, OH.

Background: BRAF mutations are oncogenic drivers which occur in about 60% of papillary thyroid cancer (PTC) as well as a significant proportion of anaplastic thyroid cancer (ATC). BRAF mutations drive MAPK signaling and treatment resistance in thyroid cancer. BRAF<sub>V600E</sub> is the most common of these mutations in PTC, and can be selectively inhibited by vemurafenib.

Methods: We used a panel of PTC and ATC cell lines to assess how the presence/absence of BRAF<sub>V600E</sub> impacts radiation sensitivity. We used radiation clonogenics, comet assays, nuclear foci formation, and western blots to determine the effect of vemurafenib on PTC and ATC cell lines.

Results: Analysis of radiation clonogenics revealed relative radioresistance in cell lines containing the BRAF<sub>V600E</sub> mutation versus wild-type. Vemurafenib inhibited MAPK signaling in V600E mutants, but showed no effect on BRAF wild-type cell lines. Vemurafenib pretreatment selectively radiosensitized BRAF<sub>V600E</sub> mutants, as assessed by radiation clonogenic assays. Neutral comet assays also showed that vemurafenib impairs DNA repair in BRAF<sub>V600E</sub> lines. Furthermore, γ-H2A.x westerns and nuclear foci staining indicated that vemurafenib pretreatment decreases the ability of cells to repair double-strand DNA breaks in cell lines containing the BRAF<sub>V600E</sub> mutation. Vemurafenib also appeared to alter the kinetics of nuclear foci formation and resolution of 53BP1 as well as Rad51 in these cell lines.

Conclusions: From our initial results, BRAF mutations appear to be associated with radioresistance in PTC and ATC cell lines. Vemurafenib selectively radiosensitizes both PTC and ATC cells through inhibition of DNA repair mechanisms. Together, these data suggest that combining vemurafenib and radiation may improve therapeutic control for BRAF<sub>V600E</sub> mutant thyroid cancers.

C32 Characterization of molecular targets of the novel platinum agent PT-112 in human colon cancer cells. Justin Q. Wang<sup>1</sup>, Tyler Ames<sup>2</sup>, Emily Arciero<sup>1</sup>, Marie-Therese Hehenberger<sup>3</sup>, Devasis Chatterjee<sup>1</sup>.<sup>1</sup>Rhode Island Hospital and The Alpert Medical School of Brown University, Providence, RI;<sup>2</sup>Phosplatin Therapeutics, LLC, New York City, NY;<sup>3</sup>University of Natural Resources and Life Sciences, Vienna, Austria.

Background: The purpose of this study was to delineate differences in the mechanism of action (MOA) and to identify unique molecular targets responsible for the antineoplastic effects of PT-112 when compared to oxaliplatin. PT-112 is a novel platinum-based chemotherapeutic agent currently under clinical development that has demonstrated superior efficacy in resistant cell lines and xenograft models. In this study we focus on the differential mechanistic effects of PT-112 and oxaliplatin treatment of HCT-116 colon cancer cells and report data derived from multiple cancer signaling pathways.

Results: Treatment of HCT-116 with the IC50 dose of PT-112 and oxaliplatin for 24 and 48h resulted in growth inhibition and apoptosis induction as measured by MTT assay, pro-caspase 8 and PARP cleavage. At equipotent doses, oxaliplatin treatment resulted in greater DNA damage as measured by H2A.X serine phosphorylation when compared to PT-112, whereas PT-112 induced markedly greater degrees of expression of several proteins in multiple pathways, including p53, p16, and FasL. PT-112's activity may derive more directly from p53 expression, when compared to oxaliplatin, as revealed by growth inhibition assays using and a p53-null HCT-116 cell line. Additionally, PT-112 triggered the cleavage of executioner pro-caspases 3, 6 and 7 to a greater extent vs oxaliplatin.
When compared to oxaliplatin, PT-112 treatment resulted in the significant inhibition of gp130 and JAK/STAT signaling and transcriptional activation mediated by IL-6. A decrease, relative to oxaliplatin, in TNF-mediated NF-κB signaling and expression of cell-cycle factors E2F3, CDK4 and CDK1, as well as cell survival protein c-FLIPshort were also observed. PT-112 was more potent than oxaliplatin in inducing the release of High-mobility group protein B1, a marker for immunogenic cell death (ICD) processes. Additionally, the inhibition of the malignant phenotype, as assessed by anchorage independent growth, was significantly greater in cells incubated with PT-112 when compared to oxaliplatin.

When treating non-malignant 1459 colon cells or biopsied benign normal colon tissue grown in culture, PT-112 did not result in apoptosis at concentrations much higher than those that were used in the HCT-116 experiments. In contrast, oxaliplatin remained equivalently potent in the non-malignant cell lines.

Conclusions: PT-112’s ability to affect numerous intracellular proteins, and the evidence of extracellular initiation of anticancer signaling, along with the apparent reduced dependence on DNA-damage, makes it an attractive and versatile compound, particularly as it relates to potential drug resistance mechanisms. PT-112’s inhibition of STAT3 activation and induction of ICD also offers the intriguing proposition of downstream immune-potentiating therapeutic effects. These results underline a unique rationale for further clinical evaluation of PT-112. In conclusion, our study has demonstrated that PT-112 treatment simultaneously regulates multiple cellular targets including apoptosis, cell survival, tumor suppressor and cell cycle proteins and pathways in a manner that is clearly differentiated from oxaliplatin.

C34 Senescence as a mechanism of resistance to the Aurora kinase and angiokinase inhibitor, ENMD-2076, in p53 mutated triple-negative breast cancer (TNBC) models. Anastasia A. Ionkina, S. Gail Eckhardt, Todd M. Pitts, Carol Sartorius, Peter Kabos, Jiyhe Kim, Aik Choon Tan, John J. Tentler, Jennifer R. Diamond. University of Colorado Denver Anschutz Medical Campus, Aurora, CO.

Background: Despite advances in targeted therapies for cancer, TNBC remains an aggressive breast cancer subtype with limited treatment options. Mutations in p53 are common in TNBC, however, the exact contribution of individual p53 mutations to response to therapy and mechanisms of acquired resistance are unknown. The purpose of this study was to characterize the activity of ENMD-2076, a multi-target Aurora kinase A and angiokinase inhibitor, against p53 mutated TNBC patient-derived tumor xenografts (PDX) and to identify differences in molecular pathways determining cellular fate in sensitive and resistant models.

Methods: TNBC PDX models harboring different p53 mutations were used for ENMD-2076 treatment studies. Athymic nude mice were injected with tumor tissue and tumor volumes were measured twice a week. When the mean tumor volumes reached 150 mm3, mice were randomized and treated with vehicle control or ENMD-2076 200 mg/kg by oral gavage daily. A subset of animals were sacrificed at Day 4, 30, and at the time of acquired resistance for correlative tissue testing which included: immunofluorescence (IF) for p53, p73, BCL2, BAX, p16, phospho Aurora A (pAA) and phospho histone H3 (pHH3); immunohistochemistry (IHC) for cleaved caspase 3 (CC3) and Ki67; H&E and staining for senescence associated beta-galactosidase (SA-β-gal) activity. Tumor growth inhibition (TGI) was calculated at Day 30 and sensitive models were treated until resistance when additional correlative tissue samples were obtained.

Results: ENMD-2076 had significant anti-tumor activity against the CU_002 and CU_005 TNBC PDX models (TGI 71.3%, p value <0.0001; TGI 66.1% p value < 0.0002, respectfully). The CU_004 TNBC PDX model was intrinsically resistant to ENMD-2076 treatment (TGI 37%, p value 0.07). In the two sensitive PDX models, we observed an increase in p53, p73, BAX and the apoptotic marker CC3. This was accompanied by a decrease in the anti-apoptotic protein BCL2 and the proliferation marker Ki67 following treatment at Day 30.
Consistent with Aurora kinase A inhibition, we detected an increase in pAA and a decrease in pH3 expression in both sensitive and resistant PDTX models following treatment at Day 4 and Day 30. At the time of acquired resistance, defined by at least doubling of tumor volumes from the maximal response, we observed loss of p73, p53, and BAX expression and an increase in p16 staining and SA β-gal activity consistent with senescence. These findings were also observed following treatment with ENMD-2076 in the intrinsically resistant CU_004 model.

Conclusions: ENMD-2076 has pro-apoptotic anti-cancer activity in a subset of p53 mutated TNBC PDX models. Sensitivity was associated with the induction of p73, which may mediate the response in the absence of functional p53. Intrinsic and acquired resistance to ENMD-2076 in TNBC PTX models was associated with loss of p73 expression and an increase in markers associated with senescence, including p16 expression and SA β-gal activity. These data support the role of senescence as a potential mechanism of resistance to Aurora kinase inhibitors in p53 mutated TNBC and support the continued development of combination therapies including with inhibitors of pathways that mediate senescence.

C35 Low-dose imatinib mesylate suppressed PDGF-BB mediated motility and invasion of gastric cancer cells. Hong Jun Kim1, Suk Young Lee1, Sang Cheul Oh1, Jun Suk Kim1, Jung Lim Kim3, Bo Ram Kim1, Yoo Jin Na4. 1Korea university medical center Guro hospital, Seoul, Korea; 2Graduate School of Medicine, Korea University College of Medicine, Korea University, Seoul, Korea; 3Graduate School of Medicine, Korea University College of Medicine, Korea University, Seoul, Korea; 4Korea University Medical Center Guro Hospital, Seoul, Korea.

Purpose: Imatinib mesylate is a powerful tyrosine kinase inhibitor that specifically targets BCR-ABL, KIT, and PDGFR kinases and is used in treatment of chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GIST), and other cancers. Although imatinib has not yet been used in gastric cancer, we verified that high-dose imatinib leads gastric cancer cell lines to apoptosis via down regulation of Akt phosphorylation in previous study. This study was undertaken to evaluate the in vitro effect of low-dose imatinib in inhibition of invasion and migration of gastric cancer cells and to define molecular mechanism underlying these effects.

Methods: This study was to determine the expression of PDGFR molecules in gastric cancer cells (four human gastric cancer cell lines; AGS, MKN28, MKN45, and SNU638) by semi-quantitative polimerase chain reaction (PCR). The effects of low-dose imatinib (≤20uM.ml) on cell viability were examined using MTT assay. The effects of imatinib and PDGF-BB on cell motility and cell invasion were studied using wound healing assay and matrigel invasion assay respectively.

Results: The results showed expression of PDGFR in all four gastric cancer cells. We measured the sensitivity of gastric cancer cell line (AGS, MKN28, MKN45, SNU638) to imatinib using MTT assay. Cell viability of low-dose imatinib treated cell was not decreased. However, cell migration and invasion was decreased with low-dose imatinib. When PDGF-BB was added, cell motility and invasion were activated. However, when both PDGF-BB and imatinib were added, that activation was not shown.

Conclusion: In conclusion, low-dose imatinib mesylate inhibits PDGF-BB mediated cell migration and invasion not decreasing cell viability. The results suggest that imatinib mesylate may be useful in the treatment of gastric cancer.
Patient-derived organoids from high-risk prostate cancer identify different biological subtypes and responsiveness to therapy. Hatem E. Sabaawy, Monica Bartucci, Mark N. Stein, Isaac Yi Kim, Joseph R. Bertino, Robert S. DiPaola, Anna Ferrari. Rutgers-The Cancer Institute of New Jersey, New Brunswick, NJ.

Clonal heterogeneity in primary prostate cancer (PC) is a well-established phenomenon from which rare subclones develop metastatic potential that seed distant sites. It is also well established that androgen deprivation increases clonal heterogeneity of PC foci, seeding between sites and progression to castration resistant (CR) disease that is poorly controlled by subsequent therapies. Genomic sequencing and expression profiling have revealed the complexity and variation of the molecular changes within foci and across patients. In spite of the heterogeneity, a number of gene/pathway alterations have been identified that discriminate molecular subtype and potential differences in response to therapies.

To study the heterogeneity of locally advanced PC in high-risk patients, identify foci with metastatic potential and differences in biologic behavior, we generated patient-derived organoids. To this end, we isolated cells from PC foci and normal epithelial counterparts from histopathologically mapped radical prostatectomy specimens and developed a 3D culture system utilizing single cell-derived tumor initiating cells (TICs) and epithelial/mesenchymal growth factors. Organoid growth peaks at 2 weeks and serially passed organoids can be maintained in culture for up to 6 months with no significant deviation from early passages. The organoids mirror the morphologic features (well-organized glandular structures with central E-Cad+ cells, peripheral Vimentin+ cells and a surrounding thick basement membrane), biomarker expression (AR and PSA) and molecular subtypes of the foci from which they were derived, including alterations in TMRSS2-Ets fusions, loss of PTEN, RB and CHD1, overexpression of Erg, BMI-1, AR and AR-Vs.

To study the biology and responsiveness of these organoids to established therapies in the androgen sensitive and castration resistant stages of PC progression, we measured single-cell-derived organoid cell proliferation, viability and secondary organoid-forming potential in response to androgen deprivation, docetaxel, abiraterone, enzalutamide, BEZ325, BMS-345541 IKB/IKK inhibitor and a novel BMI-1 inhibitor. The results show that androgen deprivation decreases cellular growth compared to control untreated organoids, although they can be passaged for up to 4 additional weeks. Monotherapy with docetaxel, abiraterone or BEZ325 significantly reduced tumor cell viability in the absence of DHT but only abiraterone and docetaxel reduced the organoid-forming potential. The BMI-1 inhibitor selectively inhibited self-renewal, and combinations of the BMI-1 inhibitor with docetaxel rescued resistance to docetaxel in CRPC-derived cells.

In conclusion, we developed a personalized and genetically defined patient-derived PC organoid model that can be utilized to identify clones with metastatic potential which may help planning systemic therapeutic interventions.

Molecular determinants of sensitivity and resistance to FGFR inhibition in FGFR2-amplified gastric cancer. Irina Babina1, Alex Pearson1, Ros Cutts1, Elizabeth Smyth2, Jian Ning1, Amanda Swain1, David Cunningham2, Nicholas C. Turner1. ICR, London, United Kingdom; 2The Royal Marsden Hospital, London, United Kingdom.

Despite improvements in diagnostics and chemotherapy regimens in gastric cancer, there is still an urgent need for novel biomarkers and second-line treatment interventions. High level FGFR2 amplification is found in ~5% gastric cancers, and responses in FGFR2-amplified gastric cancer have been observed in a phase II study of FGFR inhibitor AZD4547 (Smyth et al ASCO 2015, NCT01795768). Here we studied tumor
progression biopsies and patient-derived xenografts (PDX) to understand the mechanisms of sensitivity and resistance in FGFR2-amplified gastric cancer.

PDX models were generated from the baseline biopsies of two Caucasian patients with junctional FGFR2-amplified tumors who had durable responses to AZD4547 in the clinic. Both PDX recapitulated the histology of the original cancers, and whole exome sequencing demonstrated 85-90% agreement mutations between the patient biopsies and the PDX tumors. Similar to the patients, both models were highly sensitive to AZD4547, with regression of -60% seen after 10 days treatment, with subsequent stability on chronic dosing.

To understand the molecular mechanisms of sensitivity to AZD4547, we profiled PDX tumors and PDX-derived spheroid cultures with phospho-RTK signaling arrays and by western blot. Although FGFR inhibition resulted in short-term suppression of PI3K-mTOR signaling, chronic exposure resulted in an increase in phospho-S6 and phospho-4EBP1 after 24 hours of treatment. Combination of AZD4547 and the catalytic mTOR inhibitor AZD2014 elicited a greater response in vitro than either drug alone. To examine adaptive response to AZD4547 we excised residual PDX tumors after 60 days treatment, demonstrating upregulated ERBB3 and insulin receptor phosphorylation in tumors adapted to long-term FGFR inhibition. A treatment break, which resumed tumor growth, re-sensitized to AZD4547, suggesting that ongoing FGFR inhibition was required to maintain the adaptive response.

Finally we examined the mechanism of acquired resistance to AZD4547. Two responding patients had progression biopsies. Paired exome sequencing demonstrated an acquired KRAS amplification in one patient, though no evident onco-mutation was identified in patient two. In parallel, long-term treatment of PDX with AZD4547 gave rise to resistant PDX tumors, the results of which will be presented at the conference.

In conclusion, we show that reactivation of mTOR signaling limits sensitivity of FGFR2-amplified tumors to AZD4547, identifying potential combination strategies to deepen response. Studies of acquired resistance are ongoing, with preliminary evidence suggesting acquisition of downstream genetic events.

C38  Novel allosteric IDH1 mutant Inhibitors for differentiation therapy of acute myeloid leukemia. Ujunwa C. Okoye-Okafor1, Boris Bartholdy1, Jessy Cartier1, Enoch Gao1, Beth Pietrak2, Alan R. Renda1,2, Cynthia Rominger1, Chad Quinn1, Angela Smallwood2, Ken Wiggall1, Alexander Reif3, Stan Schmidt3, Hongwei Qi3, Huizhen Zhao3, Gerard Joberty2, Maria Faeth-Savitski2, Marcus Bantscheff2, Gerard Drewes2, Chaya Duraiswami2, Pat Brady2, Swathi-Rao Narayanagari2, Ileana Antony-Debre1, Kelly Mitchell1, Heng Rui Wang1, Yun-Ruei Kao1, Maximilian Christopeit1, Luis Carvajal1, Laura Barreyro1, Elisabeth Paitetta1, Britta Will1, Nestor Concha2, Nicholas D. Adams3, Benjamin Schwartz2, Michael T. McCabe3, Jaroslav Maciejewski4, Armit Verma1, Ulrich Steidl1, 1Albert Einstein College of Medicine, Bronx, NY; 2Department of Molecular Discovery Research, GlaxoSmithKline, Collegeville, PA; 3Cancer Epigenetics Discovery Performance Unit, GlaxoSmithKline, Collegeville, PA; 4Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH.

Mutations in the isocitrate dehydrogenase 1 (IDH1) gene are known driver mutations in acute myeloid leukemia (AML) and other cancer types. AML is hallmarked by a differentiation block and patient outcomes remain poor, especially for patients above 60 years of age who typically do not tolerate high dose chemotherapy and stem cell transplantation, leading to cure rates below 20%. Hence the development of novel targeted therapies for treatment of AML subtypes are required. Of note, inhibitors of mutants of the closely related IDH2 gene as well as IDH1 have recently been described and show promising pre-clinical and
early phase clinical activity. However, the specific molecular and functional effects of IDH1 inhibitors in AML, including in primary patients’ cells, have not been reported yet.

Here, we report the development of novel allosteric inhibitors of mutant IDH1 for differentiation therapy of acute myeloid leukemia. A high-throughput biochemical screen targeting an IDH1 heterodimer composed of R132H and WT IDH1 led to the identification of a tetrahydropyrazolopyridine series of inhibitors. Structural and biochemical analyses revealed that these novel compounds bind to an allosteric site that does not contact any of the mutant residues in the enzymes active site and inhibit enzymatic turnover. The enzyme complex locked in the catalytically inactive conformation inhibits the production of the oncometabolite 2-hydroxyglutarate (2-HG). In biochemical studies, we observed potent inhibition of several different clinically relevant R132 mutants in the presence or absence of the cofactor NADPH, accompanied by significant decrease in H3K9me2 levels.

Treatment of primary IDH1 mutant AML patients’ cells ex vivo uniformly led to a decrease in intracellular 2-HG, abrogation of the myeloid differentiation block, increased cell death and induction of differentiation both at the level of leukemic blasts and immature stem-like cells. Allosteric inhibition of IDH1 also led to a decrease in leukemic blasts in an in vivo xenotransplantation model. At the molecular level, enhanced reduced representation bisulfite sequencing showed that treatment with allosteric IDH1 inhibitors led to a significant reversal of the DNA cytosine hypermethylation pattern induced by mutant IDH1, accompanied by gene expression changes of key sets of genes and pathways, including "Cell Cycle", "G1/S transition", "Cellular growth and proliferation", and "Cell death and survival".

Taken together, our findings provide novel insight into the effects of inhibition of mutant IDH1 in primary AML patients’ cells and open avenues for future investigations with these and other novel allosteric inhibitors for targeting IDH1 mutants in leukemia and possibly in other cancers.

Chemistry

C39 First-in-class inhibitors of the putatively undruggable DNA repair target Poly(ADP-ribose) glycohydrolase (PARG). Bohdan Waszkowycz, Dominic James, Ben Acton, Emma Fairweather, Sam Fritzl, Niall Hamilton, Nicola Hamilton, Sarah Holt, James Hitchen, Colin Hutton, Stuart Jones, Allan Jordan, Alison McGonagle, Daniel Mould, Helen Small, Kate Smith, Alexandra Stowell, Ian D. Waddell, Donald Ogilvie. CRUK Manchester Institute, Manchester, United Kingdom.

Poly(ADP-ribose) glycohydrolase (PARG) is the only enzyme known to catalyse hydrolysis of the O-glycosidic linkages of ADP-ribose polymers, thereby reversing the effects of poly(ADP-ribose) polymerases (PARPs). PARG depletion, using RNAi, results in several effects such as PAR chain persistence, progression of single- to double-strand DNA lesions and NAD+ depletion. Given these findings, inhibition of PARG with a small molecule agent offers a potential opportunity to interfere with DNA repair mechanisms and induce cell death in those cells with increased susceptibility to DNA damage, such as tumour cells.

Previous efforts to develop small molecule inhibitors of PARG activity have generally been hampered by poor physicochemical properties, off-target pharmacology and a lack of cell permeability, leading some to suggest that PARG may be undruggable.

In contrast, we have now developed a series of first-in-class PARG inhibitors which display drug-like properties and attractive pharmacokinetic parameters. These compounds have proved to be useful biological tool compounds.
Moreover, displaying selective activity in both biochemical and, more importantly, cellular assays of PARG function, these derivatives have allowed an exploration of the phenotypes resulting from reversible, pharmacological PARG inhibition in both in vitro cell panels and in vivo models. Furthermore, our initial bioinformatic analysis suggests that deficiency of a known tumour suppressor confers sensitivity to PARG inhibition, suggesting patient populations that will potentially benefit from PARGi therapies.

C40 Inhibition of Mcl-1 through covalent modification of a non-catalytic lysine side chain. Qibin Su, Gizem Akçay, Neil Grimster, Matthew Belmonte, Philip Rawlins, Michelle Lamb, Alexander Hird, Brian Aquila. Astrazeneca, Waltham, MA.

Myeloid cell leukemia 1 (Mcl-1), a potent anti-apoptotic protein of the BCL2 family, has been studied as a key resistance factor in human cancers. Restoring apoptotic signals by inactivating Mcl-1 protein interactions with small molecule inhibitors has been intensively pursued as targets for cancer therapeutics. We herein describe our effort towards the structure-based design, synthesis and evaluation of first potent, covalent binders of Mcl-1. The resulting inhibitors specifically modified a non-catalytic lysine residue with high level of Mcl-1 potency in biochemical and cell based assays. Our covalent inhibitors could provide potent probes to interrogate Mcl-1 dependent cancer biology.

Clinical Trials

C41 A phase 1 study of OMN54 in patients with advanced malignancies. Daniel Renouf1, Christian Kollmannsberger1, Kim Chi1, Stephen Chia1, Anna Tinker1, Teresa Mitchell1, Stephen Lam1, Teresa Joshi2, David Kwok1, John Ostrem2, Simon Sutcliffe4, Karen A. Gelmon1. 1BC Cancer Agency, Vancouver, BC, Canada; 2Omnitura Therapeutics, Redwood Shores, CA; 3BPI Biopharmaceutical Research, Vancouver, BC, Canada; 4Omnitura Therapeutics, Vancouver, BC, Canada.

Purpose: With the increasing interest in natural products as therapeutics, we performed a Phase I open label study of OMN54 in patients with advanced malignancies to determine toxicity, maximum tolerated dose (MTD), dose limiting toxicities (DLT), and pharmacokinetics (PK). OMN54 is a multitargeted agent prepared from three Chinese botanical sources: Ganoderma lucidum, Salvia miltiorrhiza, and Scutellaria barbata, each with long histories of use as single agents.

Methods: Eligible patients (pts) were ≥ 18 years with advanced solid tumor malignancies, able to swallow oral capsules, ECOG performance status ≤ 2, measurable disease as defined by RECIST 1.0, and adequate organ function.

Results: 22 pts were enrolled in 6 dose levels, 2 at daily and 4 with twice daily dosing ranging from 1 to 5 gm orally per day; all evaluable for toxicity and 20 for response. Most common cancers included colorectal (13 pts), non small cell lung (3 pts), and ovarian (2 pts). 5 pts patients completed Cycle 1, 9 pts Cycle 2, 3 pts Cycle 3 and 1 pt each completed Cycles 4, 5, and 8. 2 pt had < 1 cycle. Only 7 AEs in 5 pts were reported as possibly related to study drug; 6 were gastrointestinal disorders, 1 a skin disorder. One GR 2 AE of vomiting was probably related to study drug. All other AEs were Grade 1. There were no treatment-related SAEs or DLTs. A recommended phase II dose (RP2D) is 2.5 g orally twice daily. PK data revealed evidence of detectable plasma total OMN54 in cohorts 1 to 6 with all 4 parent drug chemical markers with plasma half-lives of 1- 2 hours and no evidence of accumulation. Preliminary evidence of biological activity was seen with stable disease for 8 months in 1 pt and 4 pts with dose responsive reductions in TGF-β, EGF & Rantes,
biomarkers of immune suppression. Significant TGF-β decreases were seen for 4 pts at doses of 2 gms daily to 2.5 gms bid including an ovarian, colorectal, fallopian tube and esophageal cancer.

Conclusion: OMN54 was well tolerated with no DLTs observed. Further studies at RP2D of 2.5 g bid orally should be done to assess activity.

C42 Safety and preliminary efficacy results of a first-in-human phase I study of the novel cancer stem cell (CSC) targeting antibody brontictuzumab (OMP-52M51, anti-Notch1) administered intravenously to patients with certain advanced solid tumors. Pamela Munster1, S. Gail Eckhardt2, Amita Patnaik3, Anthony F. Shields4, Anthony W. Tolcher5, S. Lindsey Davis2, John V. Heymach5, Lu Xu6, Ann M. Kapoun6, Leonardo Faoro6, Jakob Dupont6, Renata Ferrarotto7, 1University of California, San Francisco, San Francisco, CA; 2University of Colorado, Aurora, CO; 3South Texas Accelerated Research Therapeutics (START), San Antonio, TX; 4Wayne State University, Detroit, MI; 5The University of Texas MD Anderson Cancer Center, Houston, TX; 6OncoMed Pharmaceuticals, Inc., Redwood City, CA.

Background: The Notch pathway plays a key role in embryonic development, the regulation of stem and progenitor cells, and is implicated in human cancer. Notch-1 (N1) signaling is activated by various mechanisms including N1 activating mutations in certain solid tumors. Brontictuzumab (BRON) is a humanized IgG2 antibody that inhibits the signaling function of N1. As such, BRON is a novel anti-cancer agent that inhibits tumor growth through direct actions on tumor cells, including CSCs, and effects on tumor angiogenesis.

Materials and methods: A phase I dose escalation and expansion study was initiated in patients (pts) with certain advanced solid tumors (cholangiocarcinoma, breast (BC), colorectal (CRC), esophageal, gastric, pancreatic, small cell lung cancers (SCLC), and adenoid cystic carcinomas (ACC)) that have rates of N1 activation between 12-50%. BRON was administered intravenously to study safety, pharmacokinetics (PK), pharmacodynamics, preliminary efficacy, and to determine the maximum tolerated dose. The trial has a biomarker (Notch1 intracellular domain (NICD)) selected expansion cohort.

Results: 44 pts have been enrolled in 8 dose escalation cohorts at doses of 0.25, 0.5, 1, and 2.5 mg/kg every 4 weeks (Q4W), and then 1.0, 1.5, 2.0, 2.5 mg/kg every 3 weeks (Q3W) with a dose expansion cohort at the MTD of 1.5 mg/kg Q3W. Tumor types included CRC (12), ACC (11), cholangiocarcinoma (7), BC (6), esophageal (3), pancreatic (2), SCLC (2), and one pt with gastric cancer. 3 pts experienced dose-limiting toxicity (DLTs) AEs with gr 3 fatigue (2.5 mg/kg Q4W), and gr 3 diarrhea (2.5 mg/kg Q3W and 2.0 mg/kg Q3W). The most frequent adverse events (AE) were: diarrhea (73%), fatigue (61%), and nausea (45%). Common grade 3 or higher AEs included diarrhea (29%) and fatigue (7%). One pt with a N1 activating mutation in ACC had partial response after 2 doses. Another patient with an inactivating FBXW7 mutation and high NICD had stable disease for more than 290 days. 5 pts had stable disease: 2 with BC, 2 with ACC, and 1 with CRC. In the dose expansion cohort (7 efficacy evaluable pts, all NICD high), 3 SDs were observed. CSC and Notch pathway markers were reduced with BRON treatment.

Conclusions: BRON is generally well tolerated. Diarrhea is the primary toxicity of this antibody. Potential early efficacy consistent with the predictive biomarker hypothesis is noted. MTD has been established and the recommended phase 2 dose is 1.5 mg/kg Q3W. Enrollment continues in the dose expansion cohort. Updated efficacy, safety, and PK results will be presented. Clinical trial information: NCT01778439.
C43 Safety, tolerability, and clinical activity of MRX34, the first-in-class liposomal miR-34 mimic, in patients with advanced solid tumors. Muhammad Shaalan Beg1, Andrew Brenner2, Jassgit Sachdev3, Samuel Ejadi1, Mitesh Borad1, Yang-Kon Kang4, Ho Lim5, T-Y Kim1, Andreas Bader8, Jay Stoudemire8, Susan Smith8, Sinil Kim8, David Hong9. 1UT Southwestern Medical Center, Dallas, TX; 2University of Texas Health Science Center San Antonio, San Antonio, TX; 3Scottsdale Healthcare Research Institute, Scottsdale, AZ; 4Mayo Clinic Cancer Center, Scottsdale, AZ; 5Asan Medical Center, Seoul, Korea; 6Samsung Medical Center, Seoul, Korea; 7Seoul National University Hospital, Seoul, Korea; 8Mirna Therapeutics, Austin, TX; 9University of Texas MD Anderson Cancer Center, Houston, TX.

Background: MRX34 is a liposomal nanoparticle formulation with an encapsulated mimic of the naturally occurring microRNA-34 (miR-34), which is lost or expressed at reduced levels in many tumors. miR-34 inhibits key oncogenic pathways by repressing multiple important oncogenes including BCL2, E2F3, HDAC1, MET, MEK1, CDK4/6, PDGFR-α/β, SIRT1, WNT1/3, NOTCH-1, β-catenin, CD44, Nanog and AXL. miR-34 also represses the expression of PD-L1 and DGKζ, thereby possibly triggering an anti-tumor immune response and cancer cell death. A multicenter Phase 1 clinical trial of MRX34 is being conducted in patients with advanced malignancies.

Methods: Eligible patients were enrolled in a standard 3 + 3 dose escalation study and were given a MRX34 starting dose of 10 mg/m2, administered intravenously (IV) twice weekly (BIW) for 3 weeks in 28-day cycles. Enrollment is ongoing into a second MRX34 dosing schedule, daily x 5 (QD x 5) in 21-day cycles. The primary objectives are to determine the maximum tolerated dose (MTD) and recommended Phase 2 dose (RP2D) for the two dosing schedules. Secondary objectives include assessments of safety, tolerability and the pharmacokinetic profile of MRX34 after intravenous dosing as well as to assess any biological and clinical activity.

Results: As of July 2015, 75 patients with advanced solid tumors were enrolled and evaluable: 44 Caucasians, 47 males, a median age of 61 years and a median of 4 prior treatments. Histologies included 30 hepatocellular carcinoma (HCC), 7 pancreatic cancer, 4 cholangiocarcinoma, 3 each of bladder, breast, colorectal, cervix and lung. The remaining 19 patients represented various other malignancies <3 patients each. The 110 mg/m2 was deemed to be the MTD for the BIW dosing schedule. The MTD has not been determined yet for the QD x 5 schedule and dose escalation is continuing. The current dose levels are 70 mg/m2 QD x 5 for HCC and 93 mg/m2 QD x 5 for other solid tumors. The following treatment-emergent, all-causality adverse events (AEs) were most frequent among 47 patients in BIW cohorts vs 28 patients in QD x 5 cohorts: fever (64% vs 61%), fatigue (57% vs 39%), chills (57% vs 36%), back pain (55% vs 36%), nausea (49% vs 39%), diarrhea (43% vs 11%), headache (34% vs 14%), vomiting (32% vs 11%), anorexia (26% vs 14%), abdominal pain (23% vs 21%), dyspepsia (23% vs 14%), insomnia (23% vs 14%), and dehydration (23% vs 4%). Most frequent grade 3/4 laboratory abnormalities were: lymphopenia (36% vs 71%), neutropenia (26% vs 36%), hyponatremia (26% vs 7%), AST elevation (26% vs 29%), leukopenia (23% vs 36%), hyperglycemia (19% vs 36%), thrombocytopenia (17% vs 29%), anemia (11% vs 21%), alkaline phosphatase elevation (11% vs 21%), and ALT elevation (9% vs 21%). One patient with HCC and another patient with melanoma achieved confirmed partial responses per RECIST.

Conclusions: MRX34 has a manageable safety profile with confirmed partial responses in two patients with advanced HCC and melanoma. Due to better tolerability, QD x 5 schedule has been chosen for further study. Following MTD determination, enrollment will soon begin into the QD x 5 expansion cohorts for selected tumors.
C44  Phase I dose escalation study of temsirolimus in combination with metformin in patients with advanced cancers. Muhammad Rizwan Khawaja, Vinu Madhusudanannair, Chaan Ng, Alpa Nick, Filip Janku, Sarina Piha-Paul, Robert Coleman, Pamela Soliman, Siqing Fu, David Hong, Daniel Karp, Vivek Subbiah, Apostolia Tsimberidou, Funda Merci-Bernstam, Karen Lu, Aung Naing. MD Anderson Cancer Center, Houston, TX; Thomas Cancer Center and Affiliated Hospitals, Charleston, WV.

Background: The mTOR inhibitors may result in upregulation of Akt, leading to undesirable cell proliferation. Metformin inhibits mTOR through different mechanisms and may enhance antitumor activity of Temsirolimus.

Objectives: Primary objectives of this open-label phase 1 trial were to evaluate safety and tolerability, and to determine the maximum tolerated dose (MTD) of Temsirolimus plus Metformin combination in patients with advanced cancers refractory to standard therapies. Secondary objective was to assess clinical tumor response with this combination.

Methods: A fixed dose of intravenous (IV) Temsirolimus 25mg weekly was combined with an escalating dose of oral Metformin (level-1: 500mg daily, level-2: 1000mg daily, level-3: 1500mg daily, level-4: 2000g daily) by utilizing a standard 3 + 3 design. Treatment was administered in 28-day cycles following an initial 2 weeks of Metformin titration during the first cycle. MTD was defined as the highest dose studied in which the incidence of dose-limiting toxicity (DLT) was less than 33%.

Results: Twenty-one patients (male/female: 7/14) with sarcoma (n=8), colorectal cancer (n=3), endometrial cancer (n=4), uterine carcinosarcoma (n=2), ovarian cancer (n=2) and other cancers (n=2) were enrolled. Median age of patients was 56 (range 18-81) years. Patients had received median of 4 (range 2-11) lines of prior systemic treatments. Three DLTs were observed including one grade 3 mucositis in a patient with dose level-1, one grade 2 pneumonitis in level-2 and one grade 3 renal failure in level-4; all 3 patients who experienced DLT were able to continue treatment after dose modification. No grade 4 or 5 toxicities were observed. Patients continued treatment for a median of 11 (range 1-99; interquartile range 8-25) weeks.

Conclusion: Combination of Temsirolimus and Metformin is feasible and well tolerated. We recommend a dose of Temsirolimus 25mg IV weekly and Metformin 2000mg orally daily administered in 28-day cycles for phase 2 study. The combination showed modestly promising effectiveness among this cohort of heavily pretreated patients. Further expansion is being conducted among patients with metastatic endometrial cancer.

C45  Phase I study of WNT974, a first-in-class Porcupine inhibitor, in advanced solid tumors. Filip Janku, Roisin Connolly, Patricia LoRusso, Maya de Jonge, Ulka Vaishampayan, Jordi Rodon, Guillem Argilés, Andrea Myers, Shu-Fang Hsu Schmitz, Yan Ji, Margaret McLaughlin, Michael R. Palmer, Jennifer Morawia-K. UT MD Anderson Cancer Center, Houston, TX; Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine, Baltimore, MD; Yale Cancer Center, Yale University, New Haven, CT; Erasmus MC Cancer Institute, Rotterdam, Netherlands; Karmonos Cancer Institute, Wayne State University, Detroit, MI; Vall d’Hebron University Hospital and Universitat Autonoma de Barcelona, Barcelona, Spain; Novartis Institutes for BioMedical Research, Translational Clinical Oncology, Cambridge, MA; Novartis Pharma AG, Basel, Switzerland; Novartis Institutes for BioMedical Research, Translational Clinical Oncology, East Hanover, NJ; Novartis Institutes for BioMedical Research, Oncology Translational Research, Cambridge, MA.
Background: Aberrant Wnt pathway activation is critical for initiation and maintenance of multiple tumor types, yet therapeutic inhibition of Wnt/β-catenin signaling has been challenged by the lack of tractable targets. WNT974 is an oral investigational agent that potently and selectively inhibits Porcupine, a membrane-bound O-acyltransferase enzyme required for Wnt secretion. Based on preclinical studies, cancers that are Wnt ligand-dependent due to upstream aberrations in the Wnt pathway (e.g. mutations in RNF43 or its homolog ZNRF3, and fusions in R-spondin [RSPO]) are predicted to be sensitive to WNT974. RNF43 is frequently mutated in microsatellite unstable colorectal, gastric, and endometrial cancers (80%, 55%, and 51% respectively), pancreatic ductal adenocarcinoma (10%), and liver fluke-associated cholangiocarcinoma (9%). ZNRF3 is mutated in adrenocortical carcinoma (21%). RSPO2 and RSPO3 fusions occur in 10% of colorectal cancers.

Methods: A Phase I clinical trial is being conducted in patients (pts) with advanced cancer to evaluate the safety and tolerability, pharmacokinetic (PK) and pharmacodynamic (PD) properties, and antitumor activity of WNT974 (NCT01351103). Dose escalation was completed in a molecularly unselected population and guided by an adaptive Bayesian logistic regression model based on dose limiting toxicities and adverse events (AEs). Pre- and on-treatment specimens (blood, skin, and tumor) were collected for PK/PD evaluation. Expansion cohort enrollment is restricted to pts with tumors harboring molecular alterations that portend Wnt ligand dependence (e.g. RNF43 mutation, RSPO fusion).

Results: As of data cut-off, March 2, 2015, 68 pts have enrolled. Sixty-six pts were treated in the dose escalation at dose ranges and schedules of 5-30 mg QD; 30-45 mg QD 4 days on, 3 days off; and 5 mg BID. WNT974 showed rapid absorption (median Tmax 1-3 h) and a mean elimination half-life of 5-8 h. Accumulation on Cycle 1 Day 15 was small (ratio <2). PK exposure was dose proportional and inter-patient variability was generally moderate. Reported AEs (>10%) suspected to be related to WNT974 included: dysgeusia (49%), decreased appetite (28%), nausea (27%), fatigue (19%), diarrhea (18%), vomiting (16%), hypercalcemia (13%), alopecia (10%), asthenia (10%), and hypomagnesemia (10%). Grade 3/4 related AEs (>2%) included: asthenia (4%), fatigue (4%), decreased appetite (3%), and enteritis (3%). Measurement of AXIN2, a β-catenin target gene, in matched skin specimens, showed Wnt pathway inhibition at a wide range of doses. A recommended dose for expansion of 10 mg QD was chosen based on PK, PD, and tolerability data; maximum tolerated dose was not determined. There was no evidence of WNT974 antitumor activity in the dose escalation. As of data cut-off, two pts had initiated study treatment in the dose expansion and one, a pt with appendiceal cancer with an RNF43 mutation, had completed the first response assessment. This pt had stable disease with tumor shrinkage of -27%. Updated clinical data will be reported.

Conclusion: WNT974 is a first-in-class Porcupine inhibitor. Preliminary clinical data suggest WNT974 has a manageable safety profile and potential for antitumor activity in a molecularly selected population. Studies to further evaluate the safety, tolerability, and antitumor activity of WNT974 as a single agent and in combination are ongoing.
C46  NSABP FC-7: A phase Ib study evaluating neratinib (N) and cetuximab (Cmab) in patients (pts) with quadruple wild-type (quad wt) (KRAS/NRAS/BRAF/PI3KCA wt) metastatic colorectal cancer (mCRC) resistant to Cmab. Samuel A. Jacobs1, James J. Lee2, Thomas J. George, Jr.3, James L. Wade, III4, Philip J. Stella5, Ding Wang6, Ashwin R. Sama7, Marc E. Buyse6, Jodi A. Kanyuch1, Ashok Srinivasan1, Kay L. Pogue-Geile1, S. Rim Kim1, Norman Wolmark9, Carmen J. Allegra1. 1NSABP Foundation, Inc., Pittsburgh, PA; 2NSABP, and The Division of Hematology-Oncology, Department of Medicine, University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA; 3NSABP, and The University of Florida, Gainesville, FL; 4NSABP, and The Central Illinois CCOP, Decatur Memorial Hospital, Decatur, IL; 5NSABP, and The Michigan Cancer Research Consortium NCORP/St. Joseph Mercy Hospital, Ann Arbor, MI; 6NSABP, and The Henry Ford Hospital, Detroit, MI; 7NSABP, and The Thomas Jefferson University Hospital, Philadelphia, PA; 8International Drug Development Institute (IDDI), Louvain la Neuve, Belgium; 9NSABP, and The Allegheny Cancer Center, Allegheny General Hospital, Pittsburgh, PA.

Background: Multiple mechanisms may account for de novo and acquired resistance to Cmab. One mechanism, HER2 amplification, promotes heterodimer formation with HER3, bypassing EGFR blockade and resulting in downstream signaling. Bertotti reported HER2 amplification rates of 2.7% in unselected CRC pts (n=2349), 13.6% in KRAS wt, Cmab-resistant pts (n=44), and 36.4% in quad wt, Cmab-resistant xenophs (n=11), suggesting that HER2 amplification is selected for by prior Cmab exposure. To test the hypothesis that dual ERBB blockade could overcome resistance to Cmab in quad wt mCRC pts, as suggested by preclinical data, we combined N, an oral small molecule tyrosine kinase inhibitor that irreversibly binds to pan ERBB receptor tyrosine kinases, with Cmab in mCRC pts who progressed on previous anti-EGFR therapy (tx).

Methods: In this phase Ib study, 15 anti-EGFR tx (Cmab or panitumumab [Pmab])-resistant pts with quad wt mCRC have been enrolled. Clinical endpoints included determination of safety and efficacy of the combination of Cmab, fixed dose 250 mg/m^2 iv weekly, and N at escalating doses of 120, 160, 200, and 240 mg/d continuously using 3+3 design. Each cycle was 28 d. All eligible pts must have had prior tx with at least oxaliplatin, irinotecan, and Cmab or Pmab, are required to have archived tumor available before initial anti-EGFR tx with quad wt profile, and agree to a research biopsy at time of enrollment (after anti-EGFR progression), ECOG PS < 2, measurable disease, and adequate hematologic and liver parameters; HER2 amplification is not required. Primary diarrhea prophylaxis with intensive loperamide is required at all dose levels for the initial 2 wks followed by titration. HER2 status is determined by IHC using image analysis-assisted microscopy to score tissues. We consider a sum of 3+ and 2+ in 40% of tumor cells as positive (pos).

Findings: The MTD of N in combination with Cmab has not been reached. Accrual to the final cohort 240 mg/d is ongoing. Of 14 pts evaluable for toxicity, 1 pt on N 240 mg/d experienced DLT of grade 3 diarrhea for >48h. One had grade 3 diarrhea at N 200 mg/d in cycle 3 for < 24 h. Grade 1-2 rash was the most common AE occurring in nearly all pts; only 1 pt had grade 3 rash. Other toxicities were mild and expected. Thus far, best response data is available on 9 pts. Using RECIST 1.1, 5 pts had stable disease (SD) occurring at N doses of 120, 160, and 200 mg/d. Three of 4 pts who were HER2 pos in their post anti-EGFR biopsy sample had SD lasting 105, 138, and 168 d; the 4th HER2-amplified pt was not evaluable. Two pts with HER2-neg tumors had SD for 42 and 97 d. Two of 4 pts with best response of progressive disease (PD) were HER2 neg, and in 2, tumor was insufficient for analysis. Of 11 paired pt samples with an adequate number of tumor cells for HER2 analysis in pre- and post-Cmab samples, 3 pts converted from HER2 neg to HER2 pos, and 1 was HER2 pos in both pre- and post Cmab samples.

Conclusion: Dual anti-ERBB therapy with Cmab and N was safe and well tolerated. Despite multiple prior therapies, SD was seen in 5 of 9 evaluable pts including 1 pt at N 120 mg/d. A trend toward longer duration...
of SD was observed in the pts who were HER2 pos. Four of 11 post-Cmab tissues (36%) were HER2 pos, including 3 who converted from HER2 neg to pos and 1 was pos pre-and post-Cmab.

Support: Puma Biotechnology, Inc.

C47 Association of folate receptor alpha (FRα) expression level and clinical activity of IMGN853 (mirvetuximab soravtansine), a FRα-targeting antibody-drug conjugate (ADC), in FRα-expressing platinum-resistant epithelial ovarian cancer (EOC) patients (pts). Lainie P. Martin¹, Kathleen Moore², David M. O’Malley³, Shelley Seward⁴, Todd M. Bauer⁵, Ramon Perez⁶, Woondong Jeong⁷, Yinghui Zhou⁸, Joseph Ponte⁹, Maurice Kirby⁸, Mohammed Al-Adhami⁹, Rodrigo Ruiz-Soto⁹, Michael Birrer⁹. ¹Fox Chase Cancer Center, Philadelphia, PA; ²University of Oklahoma, Oklahoma City, OK; ³Ohio State University, Columbus, OH; ⁴Wayne State University, Detroit, MI; ⁵Sarah Cannon Research Institute, Nashville, TN; ⁶University of Kansas Medical Center, Kansas City, KS; ⁷Institute for Drug Development, San Antonio, TX; ⁸ImmunoGen, Waltham, MA; ⁹Massachusetts General Hospital, Boston, MA.

Background: IMGN853 is a FRα-targeting ADC comprising a FRα-binding antibody conjugated with the potent maytansinoid, DM4, a tubulin-targeting agent. FRα is a membrane protein that is highly expressed in many solid tumors, particularly epithelial ovarian cancer (EOC), endometrial cancer and lung adenocarcinoma. An immunohistochemistry (IHC) assay was developed to support the FRα-expression based patient selection strategy for the clinical development of IMGN853. The assay was optimized to detect a broad dynamic range of FRα expression, allowing discrimination among weak (1+), moderate (2+) and strong (3+), levels of expression (AACR 2015 Zhao J et al). In the EOC expansion cohort of the ongoing phase 1 trial (ASCO 2015 Moore K, et al), 80% of the patients with platinum-resistant EOC screened were found to meet the FRα expression inclusion criteria of ≥ 25% of cells with at least moderate expression.

Methods: Here we report the preliminary analysis evaluating the association of FRα expression with overall response rate [partial response (PR) or complete response (CR)] for the first seventeen evaluable patients with platinum-resistant EOC who received IMGN853 at 6.0 mg/kg (adjusted ideal body weight) IV q3wk. Patients were grouped based on their FRα expression into low (25-49% of cells with ≥ moderate expression), medium (50-74% of cells with ≥ moderate expression) and high (≥ 75% of cells with ≥ moderate expression) groups. Analysis to assess the association between expression level and clinical response was performed. Results: Of the 17 patients in the analysis, 2/17 (12%) had low, 5/17 (29%) had moderate and 10/17 (59%) had high FRα expression. In the overall cohort, clinical response was observed in 9/17 patients (1 CR and 8 PRs), for an objective response rate (ORR) of 53%. When the cohort of 17 patients was grouped based on FRα expression, clinical response was observed in 0/2 low, 1/5 moderate and 8/10 high expression patients. The majority of adverse events were CTCAE grade 1 or 2, with diarrhea, blurry vision, cough, fatigue, decreased appetite, neuropathy and nausea reported in > 20% of patients. Conclusion: In these heavily pretreated platinum-resistant ovarian cancer patients, IMGN853 demonstrates promising preliminary clinical activity, with an ORR of 53% in the overall cohort and 80% in the high FRα expression subset. Preliminary analysis suggests that FRα-expression correlates well with IMGN853 activity. The association of FRα expression with IMGN853 activity will continue to be assessed as the phase I trial continues to enroll patients in the expansion cohort.
C48  **BIP (Bergonie Institute profiling) program: Fighting cancer by matching molecular alterations and drugs in early phase trials.** Maud Toulmonde, Thomas Grellety, Celine Auzanneau, Yec'han Laizet, Kevin Tran, Anne Floquet, Delphine Garbay, Jacques Robert, Isabelle Hostein, Isabelle Soubeyran, Antoine Italiano. Institut Bergonie, Bordeaux, France.

Background: BIP is an institution-wide permanent screening program started in 2014 to identify patients (pts) referred to Institut Bergonié (Bordeaux, France) with somatic alterations that can be matched to targeted therapies in early phase clinical trials. Methods: Pts with advanced solid tumors and with ECOG performance status $\leq 2$ were eligible. Tumor DNA was isolated from a FFPE archived sample when available or from a fresh tumor biopsy. DNA analysis was performed by next generation sequencing (NGS) using a panel of up to 287 genes and by comparative genomic hybridization (CGH). Results for each patient were discussed during a weekly multidisciplinary molecular tumor board in order to assess the eligibility of the patient to early phase clinical trials. Results: From Jan 1 2014 to June 30 2015, 542 pts were enrolled with median 2 prior treatments for advanced disease (range 0-9). The main tumor types were: lung (19.2%), colorectal (16.2%), breast (13.3%), ovarian (11%), and sarcomas (10%). Median age was 61 years (range 18-84). In 28 cases (5%) molecular analysis failed mainly because of insufficient tissue. The median time from first referral to reporting was 9 weeks (range 1-36). The 20 genes most frequently altered were TP53, CDKN2A, PTEN, CDKN2B, PIK3CA, MYC, ARID1A, KRAS, RBI, EGFR, ERBB2, FGFR1, APC, RICTOR, ZNF703, ATM, BRAF, NF1, FGFR3 and CCND1. Among the patients included between Jan 1 2014 and January 31 2015 (n=286), 176 patients (68%) of patients had at least one genetic alteration that was considered actionable by the molecular tumor board. 85 patients (29.7%) were included in a clinical trial with a median delay of 17 weeks between first referral and date of treatment onset. The treatment was matched with the tumor profile in 49 cases (17%). The main reasons for non-inclusion in a clinical trial despite the identification of an actionable mutation were: non progressive disease on the current line of treatment (31.5%), general status deterioration (26%), death (13%), clinical trial not available (13%), screening failure (6.5%), lost of follow-up (5.5%), and patient refusal (4.5%). 79 patients were evaluable for response according to RECIST 1.1. The disease control rate (objective response + stable disease) was 47% for patients included in clinical trials matched with the tumor profile versus 53% (p=0.9) for the group of patients included in other clinical trials The median progression-free survival was 3.6 months (95 CI 1.8-5.3) versus 3.6 months (95 CI 0.9-6.3) (p=0.5). Conclusions: Extensive molecular profiling by using high-throughput techniques is feasible in routine practice, allow identification of actionable mutations in the majority of cases and can be used to enroll patients in early phase trials matched to their tumor genotype.

C49  **Phase 1 study of CB-839, a first-in-class, orally administered small molecule inhibitor of glutaminase in patients with refractory solid tumors.** Funda Meric-Bernstam1, Angela DeMichele2, Melinda L. Telli3, Pamela Munster4, Keith W. Orford5, George D. Demitri6, Gary K. Schwartz7, Othon Iliopoulos8, James W. Mier9, Taofeek K. Owonikoko10, Mark K. Bennett6, Manish R. Patel11, Jeffery R. Infante12, James J. Harding13. 1MD Anderson Cancer Center, Houston, TX; 2University of Pennsylvania, Philadelphia, PA; 3Stanford University, Stanford, CA; 4University of California, San Francisco, San Francisco, CA; 5Calithera Biosciences, Inc., South San Francisco, CA; 6Dana-Farber Cancer Institute, Boston, MA; 7Columbia University Medical Center, New York, NY; 8Massachusetts General Hospital, Boston, MA; 9Beth Isreal Deaconess Medical Center, Boston, MA; 10Emory University, Atlanta, GA; 11Florida Cancer Specialists, Sarasota, FL; 12Sarah Cannon Research Institute, Nashville, TN; 13Memorial Sloan Kettering Cancer Center, New York, NY.

Background: CB-839 is a first-in-class highly selective inhibitor of glutaminase (GLS), a key enzyme in the utilization of glutamine by many cancer cells. Inhibition of GLS blocks the ability of tumor cells to use glutamine as a source of energy and anabolic building blocks. CB-839 has broad activity, inducing cell death
or growth arrest in triple negative breast cancer (TNBC), KRAS-mutant non-small cell lung cancer (NSCLC), clear cell renal cell cancer (RCC), and mesothelioma cell lines. GLS inhibition with CB-839 has also been shown to synergistically combine with several standard of care (SOC) chemotherapies and signal transduction inhibitors.

Methods: CX-839-001 is an ongoing Phase 1 study of escalating doses of CB-839 given continuously in 21 day cycles in advanced and/or treatment-refractory solid tumor patients (pts) to evaluate safety and tolerability, and to identify the recommended Phase 2 dose (R2PD). It was initially given three times daily (TID) without food, but based on PK and safety data, the drug is now being given twice daily (BID) with meals. Pharmacokinetics (PK) is monitored on Days 1 and 15; pharmacodynamics is measured in platelets and in tumor biopsies. Pts are currently being enrolled at the RP2D, 600 mg BID, with the tumor types noted above as well as pts receiving CB-839 in combination with SOC agents, including paclitaxel in TNBC, docetaxel in KRAS-mutant NSCLC, erlotinib in EGFR-mutant NSCLC, and everolimus in RCC.

Results: Safety data from 85 pts enrolled in the trial revealed a low rate of Gr3/4 AEs, with 9.4% (8/85) of pts experiencing a Gr3/4 AE related to CB-839. One DLT occurred on study, a drug-related Gr3 creatinine elevation in a patient with diabetic nephropathy at the 250 mg TID dose level. Reversible, asymptomatic Gr3 elevations in transaminases occurred mostly on the TID schedule in 6/32 pts (18.8%), with only one Gr3 event occurring among 53 pts (1.9%) receiving the BID regimen. Clear exposure-dependent inhibition of GLS in platelets was seen 4 hr after the first dose of CB-839, with >90% inhibition at PK trough for most pts at the RP2D.

A total of 93 pts have been enrolled on the monotherapy cohorts of the trial as of 24 July 2015, including 32 pts on TID dosing (100-800 mg) and 61 pts on BID dosing (600-1000 mg). Radiographic stable disease (SD) was observed in 6 (19%) of 31 efficacy-evaluable pts receiving CB-839 on the TID schedule and 17 (40%) of 43 efficacy-evaluable pts on the BID schedule. Pts with SD have been on study for a median of >5 cycles (range 3-18); 15 (65%) of these pts remain on treatment. Seven of 11 (64%) evaluable RCC pts on the BID dose schedule showed SD, 5 of whom remain on study. A TNBC patient has maintained SD for more than one year and has had a 23% reduction in tumor burden. GLS expression was evaluated by IHC and found to be moderate to high in most patient tumor samples. Additional correlative studies will also be presented.

Conclusions: CB-839 has been well tolerated when administered BID with food at and above doses that produced robust inhibition of GLS in platelets and in tumors. Evidence of prolonged SD in pts receiving single agent CB-839, together with strong preclinical data in combination with SOC agents including paclitaxel, everolimus and erlotinib, provides a clear rationale for continued evaluation of CB-839 in several tumor types, as a single agent and in combination with SOC therapies.

C50 Phase I dose escalation study of the oral multi-kinase VEGF inhibitor regorafenib and the anti-EGFR monoclonal antibody cetuximab in patients with advanced solid tumors including colorectal cancer. Vivek Subbiah1, David S. Hong1, Behrang Amini1, Sarina Piha-Paul1, Joanna Grace Fernandez2, Siqing Fu1, Apostolia M. Tsimeridou1, Aung Naing1, Filip Janku1, Daniel D. Karp1, Michael Overman1, Cathy Eng2, Scott Kopetz1, Funda Meric-Bernstam1, Gerald S. Falchook2. 1UT MD Anderson Cancer Center, Houston, TX; 2Sarah Cannon Research Institute at HealthONE, Denver, CO.

Background: The combination of multi-kinase VEGF inhibitor regorafenib and anti-EGFR monoclonal antibody cetuximab overcame intrinsic and acquired resistance in both EGFR-sensitive and EGFR-resistant preclinical models of colorectal cancer (CRC). (Clin Cancer Res; 21(13); 2975-83)
Methods: We designed a phase I study to determine the safety, maximum tolerated dose (MTD), recommended phase II dose (RP2D), and dose-limiting toxicities (DLTs) of the oral multi-kinase inhibitor regorafenib and the anti-EGFR monoclonal antibody cetuximab in patients (pts) with advanced cancer including metastatic colorectal cancer. Tumor responses were assessed using RECIST v1.1.

Results: Twenty seven pts were enrolled between May 2014 and August 2015. 22 (81%) pts were evaluable for toxicity and response. 5 heavily pre-treated pts were not evaluable for DLTs because they discontinued the trial before the end of the DLT window, mainly because of disease progression. 21 pts were treated at dose level 1 (cetuximab IV 200 mg/m2 followed by 150 mg/m2 weekly + regorafenib 80 mg daily) and 6 pts at dose level 2. MTD was exceeded at dose level 2 (cetuximab IV 200 mg/m2 followed by 150 mg/m2 weekly + regorafenib 120 mg daily), with 2 DLTs observed, including G3 thrombocytopenia (n=1) and G3 thrombocytopenia with intraperitoneal bleeding (n=1). The most common adverse events observed in all patients across both dose levels tested included G1-2 rash, G1-2 hypomagnesaemia, G1 myalgia, G1 fatigue, G1 nausea/vomiting. In addition other AEs included G2 hand-foot syndrome (n=5), and G2 hypertension (N=2). One patient with KRAS wt CRC achieved a partial response (PR) (46% decrease) lasting 15 months who was previously resistant to cetuximab. Another KRAS wt CRC patient with a hyper-mutated genotype [Lynch syndrome (MSI High), both BRCA1 and 2 mutations, FGFR3 mutation] achieved stable disease (SD) for > 10 months. 10 patients (37%) achieved SD>4 months or PR that included renal cell carcinoma (n=1), EGFR mutant glioblastoma multiforme (n=1), squamous cell cancer (n=1) and carcinoma of unknown primary (n=1).

Conclusions: The combination of cetuximab and regorafenib was well tolerated at doses of cetuximab IV 200 mg/m2 followed by 150 mg/m2 weekly, with regorafenib 80 mg daily. Anticancer activity was observed in patients with wild type colorectal cancer.

DNA Repair and Modulation

C51 Chemosensitisation to cisplatin by STAT3 inhibitors is mediated through the inhibition of DNA interstrand crosslink unhooking. Helen Valentine, Emily Dixon, John A. Hartley, Konstantinos Kiakos. UCL Cancer Institute, UCL, London, United Kingdom.

Signal Transducer and Activator of Transcription 3 (STAT3) is constitutively activated in many cancer cell lines, leading to survival, proliferation, angiogenesis and metastasis. The inhibition of STAT3 by natural or synthetic compounds has been shown to sensitize cancer cell lines to the chemotherapy agent cisplatin, however the molecular mechanisms contributing to this chemosensitisation have not yet been fully elucidated. Therefore we investigated the effect of STAT3 inhibition on cisplatin-induced DNA damage and key DNA repair factors.

STAT3 inhibitors stattic and curcumin were investigated in combination with cisplatin using the Suphorrhodamine B cell growth inhibition assay in the DU145 prostate cancer and A549 non-small cell lung cancer cell lines. Combination treatments result in a significant (P<0.0001) reduction in cisplatin GI50 values. These combinations were synergistic as determined by combination index value analysis using the software Calcusyn, confirming that inhibition of STAT3 enhances the cytotoxic effect of cisplatin. Using a modification of the alkaline comet assay we observed that STAT3 inhibitors significantly prevent the unhooking step of repair of cisplatin induced interstrand crosslinks (ICLs). Treatment with cisplatin alone induces formation of ICLs which peak at 9 hours and unhook by 24 hours post cisplatin exposure in both cell lines. Pre-treating cells with 12μM stattic or 48μM curcumin followed by cisplatin results in an equivalent peak formation of ICLs at 9 hours, however 24 and 48 hours post cisplatin no unhooking is observed. Therefore both STAT3
inhibitors used in this study block the unhooking stage of ICL repair. Using RT-PCR and immunoblotting we show that treatment with STAT3 inhibitors dose-dependently down-regulates EME1, BRCA1 and FANCD2 mRNA and protein levels. These three DNA repair proteins have been shown to be involved in the early stages of ICL repair and therefore down-regulation of these genes may be responsible for the impairment of ICL unhooking seen with STAT3 inhibition.

As the effects observed here have been replicated with two STAT3 inhibitors of differing chemical structure, this suggests that STAT3 is directly involved in the regulation of cisplatin-induced ICL repair through modulation of key ICL repair genes. We propose this as a contributing mechanism to the synergy reported between STAT3 inhibitors and cisplatin. The data presented here will inform the design of future drug combination strategies.


Background: PARP inhibitors (PARPi) are under clinical investigation as anticancer therapies in several indications with a focus on monotherapy in tumors with homologous recombination deficits and on combination regimens with DNA damaging chemotherapy. The combination activity of PARPi with DNA alkylating agents is due in part to trapping of PARP1 onto base excision repair intermediates. Recent studies in mice indicate that potent trapping activity limits the tolerability of PARPi in this setting. While trapping has not been directly demonstrated with PARP inhibitors alone, genetic and correlative data suggest that it may be important in the monotherapy setting as well. The objective of this study was to further delineate the role of trapping in single-agent cytotoxicity of PARPi in cancer cells and healthy bone marrow.

Methods: Cytotoxic potency was determined for cancer cells using CellTiter-Glo and for erythroid and myeloid progenitors using colony forming unit (CFU) assays. PARP1 trapping was evaluated by cellular fractionation, TR-FRET and BLI. PARPi binding affinities and off-rates were determined via SPR. Cellular PAR levels were determined by ELISA.

Results: Talazoparib, rucaparib and the novel PARPi A-934935 bound to PARP1 with similar affinities and off-rates and inhibited cellular PAR synthesis with identical potencies. Despite these similarities, talazoparib was a more potent trapping agent. Consistent with the increased trapping activity, talazoparib was >10-fold more potent than rucaparib and A-934935 at inhibiting proliferation of erythroid progenitors and >50-fold more potent in myeloid progenitors. Similar differences were observed in a panel of human cancer cell lines including BRCA1-/- breast cancer and small cell lung cancer.

Conclusions: All NAD+-competitive PARPi elicit PARP1 trapping, but different inhibitors display varying degrees of resolution between trapping and cellular PAR depletion. Understanding the relative importance of these mechanisms to the tolerability and efficacy of PARP inhibitors is of critical importance. Myelosuppression is a commonly observed toxicity in PARPi clinical trials. In the present study we used ex vivo bone marrow CFU assays to model myelosuppression. The IC50s observed were within the range of exposures observed at recommended phase II doses in monotherapy clinical trials, highlighting the clinical relevance of this experimental approach. Our data reveal that compounds inhibiting cellular PAR synthesis with equal potency span a broad range of potency in BM-CFU assays in a manner predicted by PARPi trapping activity. These results suggest that PARPi trapping is a primary driver of myelosuppression by PARP inhibitors. Our observation that trapping drives cytotoxicity to a similar extent in bone marrow and cancer cells sensitive to single-agent PARP inhibition suggests that PARPi trapping activity is not
advantageous with respect to the therapeutic index of PARPi in a monotherapy setting. This is consistent with observations related to the therapeutic index of PARP inhibitors in the clinic. These results also have important implications for selection of a PARPi for combination regimens where trapping is not required for efficacy and in which myelosuppression may be a concern, such as those containing platinum drugs or topoisomerase I inhibitors.

C53 Homologous recombination deficiency (HRD) of high grade serous ovarian tumors from the NOVA Phase III clinical study. Keith Wilcoxen1, Christopher Neff2, Victor Abkevich2, Joshua Timothy Jones2, Kathryn Kolquist2, Michael Mirza2, Jerry Lanchbury2, Keith Mikule1, Shefali Agarwal1, Anne-Renee Hartman2, Alexander Gutin2, Kirsten Timms1.1Tesaro Inc, Waltham, MA; 2Myriad Genetics, Inc., Salt Lake City, UT.

Background: Genome-wide analysis was conducted on tumors obtained from patients enrolled in the NOVA study, a Phase 3 clinical trial evaluating the PARP inhibitor niraparib as a maintenance treatment in patients with platinum-sensitive ovarian cancer. Homologous recombination deficiency (HRD) and mutations in DNA damage repair genes were evaluated.

Material and methods: DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue and used to create libraries that were hybridized to a custom Agilent SureSelect capture array carrying probes for 54,091 single nucleotide polymorphism sites distributed across the human genome, as well as probes targeting 43 genes involved in DNA repair, including BRCA1 and BRCA2. The captured and enriched DNA was sequenced on an Illumina HiSeq 2500 sequencer. Sequences covering SNP positions were used to generate allelic imbalance profiles. Measures of genomic instability, including determination of an HRD score (integer value of 0-100), were calculated using allelic imbalance profiles and determination of loss of heterozygosity (LOH) by allele-specific copy number (ASCN). A previously identified HRD threshold score of 42 was used to define HRD positivity in the absence of a BRCA mutation.

Results: The NOVA study is a Phase 3, multicenter, randomized, double-blind, placebo-controlled study of niraparib as maintenance therapy in ovarian cancer patients who have either gBRCAmut or a tumor with high-grade serous histology and who have responded to their most recent chemotherapy containing a platinum agent. Tumor BRCA mutational status, HRD score and genomic sequencing of 43 DNA repair genes were obtained from tumor samples from both gBRCAmut and non-gBRCAmut cohorts. In the gBRCAmut cohort, HRD analysis of the tumor confirmed the presence of a deleterious or suspected deleterious mutation in all cases. In addition, an HRD score ≥ 42 and the presence of a deleterious mutation in TP53 with loss of heterozygosity (LOH) were observed in nearly all tumors. In the non-gBRCA cohort, somatic BRCA mutations were observed in approximately 13% of tumors, and approximately half of tumors with no evidence of a BRCA mutation had a high HRD score. In both cohorts, the use of three scoring algorithms (LOH, telomeric allelic imbalance [TAI], large-scale state transitions [LST]), was more predictive of BRCA mutational status than LOH alone. Additional genomic sequencing identified deleterious mutations with LOH in DNA repair genes, such as BRIP1, CDK12, RAD51C, PTEN, and RAD51D, with many tumors exhibiting multiple deleterious mutations.

Conclusions: High grade serous ovarian cancer is characterized by a high degree of genomic instability. Genomic analysis in the clinical setting is able to identify patients with both germline and somatic BRCA mutations, in addition to BRCAwt tumors with other genetic defects that may be sensitive to agents exploiting deficiencies in HR.
C54  Combination of PARP inhibitor talazoparib with etirinotecan pegol exhibits synergistic anti-tumor effect in non-BRCA preclinical cancer models. Ying Feng1, Yuqiao (Jerry) Shen1, Leonard E. Post1, Steve Doberstein2, Deborah Charych2, Ute Hoch2. 1BioMarin Pharmaceutical Inc., Novato, CA; 2Nektar Therapeutics, South San Francisco, CA.

Introduction: Talazoparib (BMN 673) is a highly potent and specific PARP1/2 inhibitor (PARPi) that has demonstrated significant clinical activity in patients with germline BRCA mutation ovarian and breast cancer, as well as in patients with small cell lung cancer. Non-clinically, combination of PARPi, including talazoparib, with Topoisomerase 1 (Top1) inhibitors, such as irinotecan, has shown synergy in BRCA1 mutant MX-1 model (Shen et al. 2013; Hoch et al., NCI-AACR-EORTC 2014). Etirinotecan pegol (NKTR-102, pegylated irinotecan) is a long-acting Top1 inhibitor, designed to provide extended drug exposure to the tumor. In preclinical models, NKTR-102 has improved efficacy and tolerability over irinotecan. We therefore hypothesized that the combination of talazoparib with NKTR-102 would be well tolerated and harness the full synergistic potential of combined Top1 and PARP inhibition.

Method: In tolerability studies, three dose levels of talazoparib (0.1, 0.2 and 0.3 mg/kg, QDx14) given with NKTR-102 (10 and 50 mg/kg irinotecan-equivalent dose, iv. Q7Dx2), or irinotecan (10, 30 and 60 mg/kg, ip, Q7Dx2) were evaluated in non-tumor-bearing nude mice (Balb/c, n=4 per group). In efficacy studies, multiple dose levels of a single agent (i.e., talazoparib, NKTR-102, or irinotecan) as well as corresponding dose combinations were evaluated in NCI-H1048 and HT-29 xenograft tumors with wildtype BRCA status (n=8-10 per group). Tumor volume and body weight were measured twice weekly.

Results: For talazoparib and NKTR-102, all combination doses were tolerated, the maximum mean body weight loss reached 6.2% in the high dose group (0.3 mg/kg talazoparib plus 50 mg/kg NKTR-102). For talazoparib and irinotecan, MTD was observed at 0.2 mg/kg BMN 673 plus 30 mg/kg irinotecan. In efficacy studies, single-agent NKTR-102 had dose-dependent anti-tumor activity against NCI-H1048 tumor, while talazoparib showed limited activity with QDx14 dosing schedule. Combination of talazoparib (0.2 or 0.3 mg/kg, QDx14) with NKTR-102 (10 mg/kg, Day 1) exhibited significantly greater anti-tumor effect than either single agent alone. In contrast, combination of talazoparib (0.2 mg/kg, QDx14) with irinotecan (30 mg/kg, Day 1) only had moderate combination effect in the NCI-H1048 model. In HT-29 model, additive anti-tumor effect was also observed for talazoparib + NKTR-102, but not for talazoparib + irinotecan under the same experimental condition.

Conclusion: Our data indicate that the combination of NKTR-102 and talazoparib is better tolerated than that of irinotecan and talazoparib, and results in stronger combination anti-tumor activity. The results support potential development of the combination in human studies.


Platinum-based therapies, both cisplatin and carboplatin, are utilized as part of first-line standard of care regimens for advanced non-small cell lung cancer (NSCLC), but overall response rate and overall survival remain limited, with a 5-year survival rate of 18%. Therefore, agents that can improve responses and survival are needed. Pevonedistat (MLN4924) is an investigational small molecule inhibitor of the Nedd8-activating enzyme (NAE) currently in Phase Ib clinical trials. Nedd8 is a small ubiquitin-like protein whose activation of
the cullin family of E3 ubiquitin ligases is dependent on NAE activity. Importantly, pevonedistat synergized with carboplatin in a cell viability assay for 6 of 20 NSCLC cell lines tested in vitro, and synergy was also detected in the triple combination of carboplatin, pevonedistat, and paclitaxel. Order of addition experiments in the A549 NSCLC cell line demonstrated a benefit of simultaneous treatment or addition of pevonedistat prior to platinum treatment, while a reduced combination benefit was observed when cisplatin was added 24-48 hrs before pevonedistat. Levels of NAE pathway inhibition were similar in pevonedistat and pevonedistat+cisplatin treated cells, indicating that a drug-drug interaction was not likely the cause of the synergy. Using patient-derived xenograft (PDX) models a combination benefit of carboplatin and pevonedistat was demonstrated in a carboplatin-insensitive model but a similar improvement in response was not observed in a carboplatin-sensitive model, adding to evidence that pevonedistat might target platinum resistance mechanisms. To evaluate the mechanism of synergy between pevonedistat and platinum, in vitro experiments with RNAi were performed in 4 cell lines. These studies identified 2 mechanisms of synergy between pevonedistat and platinums in vitro. Depletion of genes within the TC-NER (transcription-coupled nucleotide excision repair) and ICR (interstrand crosslink repair) pathways reduced the synergy between pevonedistat and platinum, with the contribution of each pathway varying by cell line. Because these pathways are also implicated in the response to single agent platinums, the results suggest pevonedistat could delay completion of platinum-induced DNA repair and therefore result in enhanced cell death. Inhibition of neddylation of the E3 ubiquitin ligase CUL4-RBX1-DDB1-ERCC8 by pevonedistat may delay the TC-NER pathway, providing a direct mechanism of resistance reversal by pevonedistat. The combination of pevonedistat with carboplatin and paclitaxel is under evaluation as part of a phase 1b trial in adult patients with solid tumors (NCT01862328).

C56 Platinum containing anti-cancer agents as targeted therapeutics. Peter M. Bruno¹, Ga Young Park², Junko Murai³, Yunpeng Liu¹, Justin Pritchard¹, Yves Pommier³, Stephen J. Lippard², Michael T. Hemann¹. ¹Department of Biology, Koch Institute for Integrative Cancer Research at MIT, Cambridge, MA; ²Department of Chemistry at MIT, Cambridge, MA; ³Laboratory of Molecular Pharmacology, Center for Cancer Research, NCI, NIH, Bethesda, MD.

Cisplatin and its analogs are one of the most widely used family of cancer chemotherapeutics. However, despite a diverse array of analogs having been synthesized over the past decades, the underlying assumption has been that they all work similarly by damaging DNA. Utilizing a unique multi-platform genetic approach we show that this is not the case, and that small modifications to the core structure of cisplatin can produce unexpected changes in mechanism of action. Specifically, we found oxaliplatin and a subset of mono-functional cisplatin analogs closely resembled transcription and translation inhibitors. This set of platinum-based agents do not rely on traditional cisplatin repair and tolerance pathways such as nucleotide excision repair, homologous recombination and inter-strand cross-link repair. Instead, we’ve demonstrated oxaliplatin treatment kills cells by eliciting a ribosome biogenesis stress response. Additionally, across a panel of human cell lines, we’ve shown that higher expression of ribosomal proteins correlates with resistance to these transcription/translation inhibitor-like platinum agents. From this analysis, and by analyzing and comparing human patient tumor expression data, we’ve concluded that oxaliplatin treatment is best applied in tumors that have developed a heightened dependence on translation. Thus, while commonly regarded as “blunt weapons”, our current arsenal of genotoxic anti-cancer agents utilizes surprisingly diverse mechanisms that suggest new paradigms for their application in patients.
C57  Discovery and development of replication protein A (RPA)-DNA interaction inhibitors for cancer chemotherapy. Navnath Gavande, Pamela S. VanderVere-Carozza, Akaash Mishra, John J. Turchi. Indiana University School of Medicine, Indianapolis, IN.

Platinum (Pt)-based chemotherapeutics exert their efficacy via the formation of DNA adducts which interfere with DNA replication, transcription and cell division and ultimately induce cell death. DNA repair of Pt-DNA adducts via nucleotide excision repair (NER) or homologous recombination repair (HRR) can substantially reduce the effectiveness of the Pt therapy, which is a major contributor for cellular resistance. Inhibition of these repair pathways, therefore, holds the potential to sensitize cancer cells to Pt-treatment and increase the clinical efficacy. Replication protein A (RPA) is a single-stranded DNA binding protein that plays a crucial role in the NER and HRR pathways apart from its role in replication and DNA damage checkpoint activation. We have pursued the development of small molecule inhibitors of the RPA-DNA interaction as a mechanism to target RPA activity and increase the efficacy of Pt-based cancer chemotherapy. We have developed an inhibitor, TDRL-551, that targets the central oligonucleotide/oligosaccharide binding folds in DNA binding domains A and B (DBD-A/B) of the 70 kDa RPA subunit. This lead compound shows synergy with cisplatin in tissue culture models of epithelial ovarian cancer (EOC) and in vivo efficacy, as a single agent as well as in a combination with platinum, in a lung cancer xenograft model. We have expanded our synthetic scheme and defined structure-activity relationships (SAR) towards optimization of TRDL-551 as an anticancer therapeutic for the treatment of lung and ovarian cancer.

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C58  Small molecule inhibitors targeting the interaction of xeroderma pigmentosum group A protein with cisplatin-damaged DNA. Pamela S. VanderVere-Carozza, Navnath Gavande, Akaash Mishra, John J. Turchi. Indiana University School of Medicine, Indianapolis, IN.

Targeting DNA repair and the DNA damage response for cancer therapy has recently gained increasing attention with inhibitors of the PI3-K-like kinases in early stage clinical trials. The utility of DNA repair inhibitors can be expanded by their use in combination treatment with DNA damaging chemotherapeutics including cisplatin. We have focused on directly targeting the DNA repair pathway responsible for repairing platinum-induced DNA damage, nucleotide excision repair (NER). We have selected the molecular target XPA, whose role is in the identification and verification of the sites of DNA damage. Clinical validation of XPA has been obtained where high XPA expression in lung, ovarian and lung cancer results in decreased efficacy of platinum therapy. We report the continued development of the X80 class of XPA small molecule inhibitors. In a two-step, iterative process we have identified critical structure activity relationships that have resulted in a 100-fold increase in potency with in vitro IC50 values below 1µM. Analyses of the SARs define the chemical and structural features that impact the interaction with XPA, cellular permeability and contribute to selectivity. Data demonstrate that the X80 class of inhibitors do not interact with DNA but directly bind the XPA protein. Recent production of a sub-fragment of the XPA protein will allow the identification of the direct binding domain to enable continued structure-based design of more potent XPA inhibitors.

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C59 Role of MTH1 (NUDT1) in cancer cell survival. Husam Alwan1, Kay Eckersley1, Louise Goodwin1, Alan Lau1, David Jones1, Jason Kettle2, Willem M. Nissink2, Jon Read2, James S. Scott2, Benjamin J.M. Taylor2, Graeme E. Walker1, Kevin M. Foote2. 1AstraZeneca, Macclesfield, United Kingdom; 2AstraZeneca, Cambridge, United Kingdom.

Human mutT homolog MTH1 (also known as NUDT1) is a purine nucleoside triphosphatase which hydrolysates oxidised nucleotides (8-oxo-dGTP and 2-OH-dATP) into mono-phosphate forms to prevent these damaged bases from being incorporated into DNA. Recent studies have suggested a key role of MTH1 in the survival of cancer cells. It was hypothesized that in cancer cells with high levels of reactive oxygen species (ROS), small molecule inhibition or loss of MTH1 would lead to accumulation of oxidised nucleotides in DNA, increased genome instability and loss of cell viability. In order to validate MTH1 as a potential cancer therapeutic target we have developed additional potent small molecule inhibitors of MTH1 and generated siRNA knock-down and CRISPR-mediated knock-out cell lines. Using these tools we evaluated the impact of either MTH1 inhibition or loss of expression on cell viability, proliferation, induction of DNA damage and cell cycle arrest across multiple cell lines in vitro. While we have confirmed that the previous study compounds (TH588, TH287, (S)-crizotinib) are potent inhibitors of MTH1 and have broad cancer cell line growth inhibition activity, we were unable to demonstrate a corresponding induction of γH2AX, 53BP1 or phospho-ATM DNA damage response (DDR). In addition none of our MTH1 inhibitors were able to show significant growth inhibitory activity across cell lines panels or induce a DDR despite being able to potently engage MTH1 in cells as evidenced by cell thermal stability assay (CETSA). Due to the lack of DNA damage induction and inconsistent anti-proliferative activity between MTH1 inhibitors, we investigated the effects of loss of protein expression using additional, independent siRNA and CRISPR knockout cell lines. Despite potent and long lasting siRNA knockdown of MTH1 protein expression in U2OS cells, we did not observe significant differences in cell viability or induction in DNA damage when compared to control cell lines. Consistent with these findings we were also able to generate SW480 clones with complete knockout of all MTH1 alleles and this did not impact growth rates when compared to parental cells. Finally, to probe the specificity of MTH1 inhibitors we assessed their ability to impact the growth of these cells which do not express MTH1 alleles and this did not impair growth rates when compared to parental cells. Here we show through the use of novel small-molecule MTH1 inhibitors, with further experiments with RNAi-mediated knockdown of MTH1 and the ability to generate stable CRISPR-mediated MTH1 knockouts, that the cellular effects previously ascribed to MTH1 inhibition may be driven through alternative mechanisms. In summary, our results do not support an essential role for MTH1 in cancer cell survival.

C60 Preclinical efficacy of the ATR inhibitor AZD6738 in combination with the PARP inhibitor olaparib. Alan Lau1, Elaine Brown1, Andrew Thomason1, Rajesh Odedra1, Victoria Sheridan1, Elaine Cadogan2, Shirlian Xu3, Andy Cui3, Paul R. Gavine3, Mark O’Connor1. 1AstraZeneca, Macclesfield, United Kingdom; 2AstraZeneca, Cambridge, United Kingdom; 3AstraZeneca, Shanghai.

The PARP inhibitor olaparib acts through both inhibition of DNA single-strand-break repair and trapping of PARP-DNA complexes creating DNA lesions which cause replication fork stalling and collapsed fork DNA breaks. Cells which have lost BRCA-dependent homologous recombination repair are highly sensitive to olaparib treatment and this has led to its approval in patients with tumors carrying BRCA mutations. In addition, ATM (Ataxia telangiectasia mutated) and ATR (Ataxia telangiectasia mutated and Rad3 related) dependent DNA repair processes are hypothesized to be important survival pathways to PARP inhibitor treatment. In pre-clinical studies cancer cells which have defects in either ATM or ATR have been shown to be sensitive to PARP inhibitors. Here we present data that the orally bioavailable ATR inhibitor AZD6738 (in
Phase-I clinical trials combine synergistically with olaparib leading to cell death and anti-tumour activity in pre-clinical models. The combinations are effective across a panel of gastric and lung cancer cell lines in vitro. In addition, results from isogenic ATM−/− knockout versus ATM+/+/+ FaDu head and neck cancer cell line pairs show enhanced combination activity in ATM knockout cells versus ATM wild-type cells. Studies in vivo show that through intermittent dosing the combination is tolerated while demonstrating significant anti-tumour efficacy and regressions across multiple human patient derived primary explant models. Together, these data support the notion of development of AZD6738 and olaparib combinations for the treatment of ATM-deficient cancers.

Drug Metabolism, Transport, and Biodistribution

C61 Non-homogeneous drug penetrance of veliparib measured in triple-negative breast tumors. Imke H. Bartelink1, Brendan Prideaux2, Gregor Krings1, Lisa Wilmes1, Pei R.E. Lee1, Byron Hann1, Jean-Philippe Coppe1, Diane Heditsian1, Lamorna Swigart-Brown1, Ella F. Jones1, Sergey Magnitsky1, Ron Keizer1, Laura Esserman1, Weiming Ruan1, Alan Wu1, Douglas Yee1, Veronique Dartois2, Denise Wolf1, Rada Savic1, Laura vantVeer1. 1University of California, San Francisco, San Francisco, CA; 2The State University of New Jersey, Newark, NJ.

Background: Veliparib, an inhibitor of Poly(ADP-ribose) polymerase (PARPi), in combination with carboplatin showed efficacy in triple negative breast cancer (TNBC) patients in the I-SPY 2 TRIAL. However ~42% of TNBC did not achieve pathologic complete response. Insufficient uptake of drug in TNBC may lead to inadequate response to PARPi. As a first step toward testing this hypothesis in patients, we quantified veliparib penetration in mouse xenograft models of TNBC.

Methods: MDA-MB-231, HCC70 or MDA-MB-436 human TNBC cells were implanted in 41 beige SCID mice. Veliparib at low dose (20mg/kg) or high dose (60mg/kg) and carboplatin (60mg/kg) was given three times daily for three days. MR images were taken at day 1. Plasma, fresh frozen and OCT embedded tissues were analyzed using Matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI) Liquid chromatography–mass spectrometry (LC-MS). Drug penetration was compared among doses and cell lines.

Results: Ex vivo veliparib concentrations quantified by LC-MS differed significantly among the tumors derived from the three cell lines. Liver and plasma concentrations were uniformly high in all mice compared to tumor and muscle tissues. Plasma pharmacokinetics in mice exhibited non-linear clearance resulting in prolonged high plasma levels at higher doses, while tumor and plasma concentrations were linearly correlated. MALDI-MSI images of tumor and muscle in 12 mice showed higher veliparib concentrations in necrotic areas compared to areas with viable tumor cells (P=0.126, Table) and higher concentrations at the rim then in the center of the tumor (P=0.046). Lower concentrations were found in MDA-MB-231 than in other cell lines (0.008). Contrast agent and veliparib accumulated near the rim of the tumors and a fast elimination of the contrast agent from the tumor correlated with relatively low veliparib tumor concentrations.

Conclusions: The spatial distribution of veliparib in TNBC depends on the dose and tumor cell biology. We demonstrated that MALDI-MSI can be used to measure veliparib penetration tumor samples, which may have potential to monitor response to PARPi therapies.
C62 Effects of rolapitant on the pharmacokinetics of dextromethorphan (CYP2D6), tolbutamide (CYP2C9), omeprazole (CYP2C19), efavirenz (CYP2B6), and repaglinide (CYP2C8) in healthy subjects. Xiaodong Wang, Zhi-Yi Zhang, Jing Wang, Sharon Lu, Sujata Arora, Lorraine Hughes, Jennifer Christensen, Vikram Kansra. TESARO, Waltham, MA.

Introduction: Rolapitant is a selective and long acting NK-1 receptor antagonist for the prevention of chemotherapy-induced nausea and vomiting (CINV). In vitro results indicated rolapitant mildly inhibited cytochrome P450 (CYP450) enzymes (2D6/2C9/2C19/2B6/2C8) at high concentrations (IC50s > 7 \(\mu\)M). The major metabolite SCH720881 did not inhibit these CYP enzymes.

This study aimed to 1) evaluate the effects of rolapitant on the pharmacokinetics (PK) of CYP probe substrates (dextromethorphan [DET] for CYP2D6, tolbutamide [TOL] for CYP2C9, omeprazole [OMP] for CYP2C19, efavirenz [EFA] for CYP2B6 and repaglinide [REP] for CYP2C8), and 2) evaluate the safety and tolerability of rolapitant co-administered with these substrates in healthy subjects.

Methods: This was an open-label, multi-part drug-drug interaction study in cohorts of 20 to 26 healthy subjects of orally-administered CYP probe substrates (Part-A: 30 mg DET; Part-B: 500 mg TOL plus 40 mg OMP; Part-C: 600 mg EFA; Part-D: 0.25 mg REP) in the absence and presence of single oral dose 180 mg rolapitant. Blood samples for determination of plasma concentration of CYP substrates and relevant metabolites were collected during 3 dosing periods in each part: 1) Period-1: CYP probe substrate alone as baseline; 2) Period-2: CYP probe substrate plus rolapitant concomitantly after a washout of probe given in Period 1 to evaluate the potential impact of rolapitant on probe substrate; and 3) Period-3: CYP probe substrate alone 7 days after the concomitant dose in Period-2 (approximating the peak time of metabolite SCH720881) to evaluate the impact of metabolite/rolapitant on probe substrate.

Results: Rolapitant inhibited CYP2D6 following concomitant dose (Period-2) and 7 days after concomitant dose (Period-3), resulting in 2.2- to 3.3-fold higher exposure (Cmax and AUC) of DET. Rolapitant did not inhibit CYP2C9 following exposure to TOL. The exposure (Cmax & AUC) of CYP2C19 substrate OMP was minimally increased by rolapitant (1.1- to 1.4-fold) and is unlikely to be clinically relevant. Rolapitant did not inhibit CYP2B6 or result in clinically relevant changes in exposure of EFA. Rolapitant did not inhibit CYP2C8 or result in clinically relevant changes in exposure of REP. There were no noteworthy adverse events or laboratory findings in any part of the study.

Conclusions: Rolapitant was well-tolerated when co-administered with CYP probe substrates. Co-administration of rolapitant increased the exposure of DET. The inhibition of CYP2D6 can last at least 7 days following single dose of rolapitant. No clinically significant interaction was observed between rolapitant and substrates of CYP2C9, CYP2C19, CYP2B6 or CYP2C8; therefore no dosing adjustment is necessary for drugs which are metabolized by these CYPs.

C63 Effects of rolapitant on the pharmacokinetics of digoxin and sulfasalazine in healthy subjects. Xiaodong Wang, Zhi-Yi Zhang, Sujata Arora, Lorraine Hughes, Jing Wang, Sharon Lu, Jennifer Christensen, Vikram Kansra. TESARO, Waltham, MA.

Introduction: Rolapitant is a selective and long acting NK-1 receptor antagonist for the prevention of chemotherapy-induced nausea and vomiting (CINV). In vitro results indicated that rolapitant inhibited P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), the efflux transporters that play important roles in drug disposition and distribution, with an IC50 of 7.4 \(\mu\)M and 4.6 \(\mu\)M, respectively. The major metabolite SCH720881 did not inhibit these transporters.
The aims of these studies were to 1) evaluate the effects of rolapitant on the pharmacokinetics (PK) and pharmacodynamics (PD) of a P-gp substrate (digoxin), and on the PK of a BCRP substrate (sulfasalazine); and 2) evaluate the safety and tolerability of rolapitant co-administered with these substrates in healthy subjects.

Methods: These were open-label, drug-drug interaction studies of orally administered substrate (0.5 mg of digoxin or 500 mg sulfasalazine, respectively) in the absence and presence of a single oral dose of 180 mg rolapitant. Cohorts consisted of 16 and 20 subjects, respectively.

For the P-gp study, blood samples for digoxin PK were collected up to 96 hours post digoxin dose in both Period 1 (digoxin alone) and Period 2 (digoxin + a single dose of rolapitant). For PD evaluation, ECGs were performed at specified time points for both Periods 1 and 2. For the BCRP study, blood samples were collected up to 30 hours after Day 1 (sulfasalazine alone), Day 3 (sulfasalazine + rolapitant) and Day 10 (sulfasalazine alone) dosing. All samples were used to evaluate the impact of rolapitant and its major metabolite, SCH720881, on the PK of the relevant substrates.

Results: Co-administration of digoxin with rolapitant increased mean digoxin Cmax value by 71% and mean AUC by 30%; trough concentrations were not impacted. There is no consistent pattern or trend to indicate a different PD profile of digoxin when administered with rolapitant. Digoxin administered either alone or in the presence of rolapitant was well tolerated.

Rolapitant inhibited BCRP resulting in an increase in exposure of sulfasalazine. Geometric mean ratios were 2.4 and 1.2 for Cmax; 2.3 and 1.3 for AUC for the comparison of Day3/Day1 and Day10/Day1, respectively. There were no noteworthy adverse events or laboratory findings in this study.

Conclusions: Rolapitant was well tolerated when co-administered with either the P-gp or BCRP substrate. Co-administration of rolapitant (180 mg) and digoxin (0.5 mg) had no clinically meaningful effect on PD response when compared with digoxin alone. Co-administration of rolapitant (180 mg) increased the exposure of sulfasalazine (500 mg) by approximately 2-fold. Monitoring for adverse reactions related to P-gp or BCRP substrates with a narrow therapeutic index may be necessary if rolapitant is concomitantly administered.

Drug Resistance and Modifiers

C65  TRK kinase domain mutations that induce resistance to a pan-TRK inhibitor. Adriana Estrada-Bernal¹, Anh T. Le¹, Brian Tuch², Tatiana Kutateladze¹, Robert C. Doebele¹.¹University of Colorado Anschutz Medical Campus, Aurora, CO; ²LOXO Oncology, San Francisco, CA.

Background: Gene fusions involving members of the NTRK family have been identified in several types of cancer. The use of TRK inhibitors in vitro and in vivo have demonstrated activity against a number of different NTRK fusions in different tumor types and most recently in a patient with an NTRK1 gene fusion (Doebele et al., Cancer Discovery 2015). Thus, the use of a pan-TRK inhibitor presents a therapeutic opportunity for multiple patient populations. LOXO-101 is an inhibitor of the TRK kinase and is highly selective for the TRKA/B/C family of kinases. Kinase domain (KD) mutations are the most common mechanism of acquired drug resistance found in patients. We therefore undertook a genetic approach to identify candidate resistance mutations in the TRK kinase domain.

Methods: We used N-ethyl-N-nitrosourea (ENU)-exposed Ba/F3-MPRIP-NTRK1, Ba/F3-PAN3-NTRK2 and Ba/F3-ETV6-NTRK3 cells to generate mutations that could allow growth of Ba/F3 cells despite the presence
of LOXO-101. Mutations identified in the initial screen were validated by cloning the mutation-bearing cDNAs back into Ba/F3 cells to evaluate their sensitivity to LOXO-101 using both proliferation assays and TRK phosphorylation by immunoblot analyses. Modeling of the mutations was performed by predicting the consequences of the amino acid substitutions within the context of a drug-bound TRK kinase domain crystal structure.

Results: We have identified three KD mutations within the TRKA kinase domain: V573M, and F589L, G667S. These mutations induce significant drug resistance in BA/F3 cells to LOXO-101 in vitro compared to the unmutated MPRIP-NTRK1. Furthermore, these mutations do not show inhibition of phosphorylation of TRKA at doses that inhibit the native MPRIP-NTRK1. The TRKA F589 position corresponds to the gatekeeper position, homologous to L1196 in ALK or T790 in EGFR, and a common position for drug resistance mutations. The TRKA G667 lies adjacent to the F589 position in the ATP/drug-bind pocket and similar resistance mutations have been observed in the ALK kinase domain following resistance to crizotinib. V573 is also positioned adjacent to both the G667 and F589 residues in the ATP-/drug-binding pocket. Although a similar mutation has been observed in vitro in ALK (V1180M), it has not yet been reported in patient tumor samples. Analysis of additional candidate resistance clones is ongoing.

Conclusion: This genetic screen identified several novel mutations in the TRK KD that may confer clinical resistance to LOXO-101. All three mutations identified to date appear to hinder binding by altering the ATP binding site of the kinase. These data should be confirmed in patients who develop clinical resistance, and may assist in the design of next generation TRK inhibitors to potentially overcome acquired resistance in patients treated with LOXO-101 or structurally similar TRK inhibitors.

C66 SPHK1 expression associated with acquired resistance to oxaliplatin in colon cancer cell lines. Hee Yeon Lee1, Myung Ah Lee1, Jin Hee Park2. 1Seoul St.Mary’s Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea; 2Cancer Research Institute, College of Medicine, The Catholic University of Korea, Seoul, Korea.

Background: Oxaliplatin is a main chemotherapeutic agent in the treatment of advanced colorectal cancer. The response rate has been reported to be over 50%, especially when combined with a target agent. However, most patients develop resistance to oxaliplatin even in responding patients, leading to cancer related death due to organ dysfunction. Unfortunately the mechanism of resistance of oxaliplatin is unclear. In the present study, we tried to identify the role of Sphingosine Kinase 1 (SPHK1) involved in the acquired resistance to oxaliplatin using colon cancer cell line.

Method: We treated HCT-116 colon cancer cell lines with gradient concentrations of oxaliplatin starting at the dose of IC50. After treated with initial dose of oxaliplatin for 48hrs, we collected the viable cell and then treated again with elevated dose of oxaliplatin. After prolonged period of exposure to low dose of oxaliplatin, we compared cell viable test and protein expression between HCT-116 wild cell and oxaliplatin treated HCT-116 cell line. We also investigated the change of response to oxaliplatin after tranfection of SPHK1 into wild type of HCT-116 cell line. The viability was determined by Neutral Red uptake assay.

Result: After prolonged repeated exposure of low dose oxaliplatin, HCT-116 cell line showed resistance to oxaliplatin and expressed new protein. We identified this new protein as SPHK1 using proteomics. Comparing the wild type and the resistance cell line, phosphorylated Akt, phosphorylated ERK, and PTEN expression were upregulated in the resistant cell line. After transfection of SPHK1 into the wild type HCT-116 cell line, response to oxaliplatin decreased comparing to the control group.
Conclusion: Based on our data, SPH1K can be a potential factor for the acquired resistance to oxaliplatin in colon cancer. It is not known yet the association between SPHK1 and PTEN in the mechanism of resistance. Currently, we are conducting experiment to identify these associations and mechanism of acquired resistance using other cell lines and human tissue.

C67 Mitochondrial dysfunction confers therapy resistance in Hep3B hepatocellular carcinoma cell line. YuSeon Han, MyeongEun Je Gal, EuiYeun Yi, YungJin Kim. Pusan National University, Busan, Korea.

Mitochondria dysfunctional cells are useful models in studies for mitochondrial diseases, apoptosis, cancer and aging, but their functional aspects have not been fully understood. Mitochondrial dysfunction (MD) is found in many type of cancer such as colorectal, liver, pancreatic, breast, prostate, ovarian cancer etc. MD has also been known to be associated with cancer malignancy, especially apoptosis resistance (therapy resistance). Using mitochondrial-depleted \( \rho^0 \) derived from Hep3B, we have tested the effects of mitochondrial dysfunction on the chemical and radiation resistance. When compared to parental cells, Hep3B/\( \rho^0 \) cells were much less sensitive to doxorubicin and sorafenib and irradiation. Hep3B/\( \rho^0 \) cells also showed increased efflux, decreased target molecules, rapid repair and DNA protection. These results suggest that mitochondrial dysfunction confers therapy resistance on the cancer cells, which could be a novel therapeutic target for cancer therapy.

C68 SLCO1B3 influences taxane-response in prostate cancer. Ellen S. de Morree1, Rene Bottcher1, Robert J. van Soest1, Ashraf Aghai1, Corrina M. de Ridder1, Alice A. Gibson2, Ron HJ Mathijsen1, Herman Burger1, Erik AC Wiemer2, Alex Sparreboom2, Wytske M. van Weerden1, Ronald de Wit1. 1Erasmus MC Cancer Institute, Rotterdam, Netherlands; 2St. Jude Children’s Research Hospital, Memphis, TN.

Introduction: Castration resistant prostate cancer (CRPC) patients are treated with docetaxel as first-line chemotherapy. Unfortunately, response to docetaxel is highly variable among patients and intrinsic or acquired resistance is common. Understanding mechanisms of resistance may help to define biomarkers to select patients for the best therapy options and prevent treatments to which they may respond poorly. We established docetaxel-resistant xenografts from chemotherapy-naïve patient-derived xenografts (PDXs) \textit{in vivo}. Next generation sequencing analysis of these chemotherapy-naïve and docetaxel-resistant PDXs revealed that SLCO1B3 expression was significantly downregulated in a subset of docetaxel-resistant tumors. As SLCO1B3 is also a known transporter of testosterone, the aim of the study was to further investigated its expression in relation with hormonal status of CRPC cells.

Methods: Androgen responsive SLCO1B3 expressing PC346C cells were used to perform uptake assays with \([^{14}C]\)-labeled docetaxel and cabazitaxel after silencing of SLCO1B3 with siRNA. The androgen resistant PC346C-DCC-G clone, created by long-term culturing of PC346C cells in androgen deprived medium, did not express SLCO1B3. PC346C-DCC-G cells were stably transfected with SLCO1B3 or with a control construct containing green fluorescent protein (GFP). MTT cell proliferation assays were performed after 10 days of exposure to docetaxel or cabazitaxel in a dose range of 0-10nM. Prostate specific antigen (PSA) production was measured with an ELISA assay after exposure of 0.1nM testosterone for 30 minutes.

Results: Silencing of SLCO1B3 in parental PC346C decreased uptake of docetaxel and cabazitaxel 2.0-fold (p=0.01) and 2.1-fold (p=0.0003), respectively. In line with this observation, SLCO1B3-transfected, overexpressing PC346C-DCC-G cells were more sensitive to docetaxel and cabazitaxel, resulting in reduced IC50-values of 1.9-fold for docetaxel and 3.5-fold for cabazitaxel compared to control transfected cells.
Moreover, PSA production in SLCO1B3-overexpressing PC346C-DCC-G cells increased 2-fold as compared to control (p=0.05) after testosterone exposure.

Conclusion: Prostate cancer cells that overexpress SLCO1B3 are more sensitive to docetaxel and cabazitaxel treatment, which could be linked to increased uptake of both taxanes. Further studies are needed to clarify the role of SLCO1B3 in the uptake of cabazitaxel into the cell. Moreover, SLCO1B3 expression affects hormonal status of prostate cancer cells as reflected by PSA production. Research is ongoing to further elucidate the role of SLCO1B3 in prostate cancer and its impact on taxane efficacy and response.

C69 Schedule-dependent synergy between the histone deacetylase inhibitor belinostat and the dihydrofolate reductase inhibitor pralatrexate in T- and B-cell lymphoma cells in vitro. Godefridus J. Peters1, Frank H. Van Gemert1, I. Kathmann1, Saskia A. Cillessen1, Gerrit Jansen1, Guru Reddy2. 1VU University Medical Center, Amsterdam, Netherlands; 2Spectrum Pharmaceuticals, Irvine, CA.

Pralatrexate (Folotyn; FOL) and belinostat (Beleodaq; BEL) have recently been registered for the treatment of patients with peripheral T-cell lymphoma (PTCL) and have promising activity in other lymphoma. FOL is a folate analog and a potent dihydrofolate reductase (DHFR) inhibitor, designed to accumulate in cancer cells via the reduced folate carrier (RFC) and retained via efficient polyglutamylation. DHFR inhibition leads to an imbalance of deoxynucleotides (depletion of dTTP and an increase in dUTP) resulting in DNA strand breaks and inhibition of DNA synthesis. BEL is a hydroxamic acid-based pan-histone deacetylase (HDAC) inhibitor that inhibits all of the zinc-dependent HDAC enzymes, with high affinity for the Class I, II and IV isozymes. HDAC inhibition results in an alteration in the degree of histone and non-histone protein acetylation, which in turn affects transcription of genes essential in cellular proliferation, cell cycle and DNA repair. In this study we investigated whether folate transporters other than RFC, i.e. folate receptor α (FRα) and the proton-coupled folate transporter (PCFT) could contribute to the efficacy of FOL. Moreover, we explored whether in combination experiments BEL had the ability to potentiate the cytotoxicity of FOL. A panel of lymphoma cell lines was used for the combination studies: the B-cell SU-DHL-4, SU-DHL-5, HT, Jeko-1 and T-cell Karpas-299 and Hut-78. RFC-mediated uptake efficiency of FOL (in competition with [3H]-methotrexate), showed a 6-fold better RFC substrate affinity for FOL, and 2-fold better than levo-leucovorin (l-LV). FOL had a very poor substrate binding affinity for FRα (>100-fold lower than folic acid and > 10 lower than l-LV) and a low affinity for PCFT (>10-fold lower than folic acid and l-LV in [3H]-LV uptake competition experiments). Sensitivity (IC50 concentrations after 72 hr exposure) to FOL varied from 2.8 (Hut-78), 5.5 (SU-DHL4 and 5), 7.4 (HT) to 20 nM (Karpas-299 and Jeko-1) and for BEL from 100 (SU-DLH-4 and 5, Jeko-1 and Hut-78) to 200 nM (Karpas-299 and HT).

The interaction between BEL and FOL was studied using the median-drug effect analysis. At a fixed ratio between the drugs based on the IC50 concentration the average combination index (CI) for all cell lines showed additivity (CI: ±1.0). In two selected cell lines (SU-DHL-4 and HT) sequential exposure (24 hr pretreatment with either BEL or FOL, followed by 48 hr to FOL + BEL), did not improve interaction (CI: 0.9-1.4). As an alternative approach a non-fixed ratio was used by exposing SU-DHL-4 and HT cells to IC25 concentrations of either BEL or FOL in combination with the other drug. Exposure to IC25 of FOL did not decrease the IC50 for BEL (CI around 1.2), but exposure to IC25 of BEL markedly increased FOL sensitivity (low CIs from 0.40-0.66). Mechanistic studies focused on induction of apoptosis, showed cleavage of caspase 8 and 9 in HT and SU-DHL-4 cells for both drugs at their IC50s, being similar in the combination setting. Moreover, at these concentrations, the drugs were shown to confer an S-phase arrest.
In conclusion, the combination of FOL and BEL showed additivity in various lymphoma cell lines, with a schedule-dependent synergism. Based on these data, proficient inhibition of HDAC activity by BEL holds promise in sensitization of tumor cells to FOL.

**C70 Differences in signalling and phenotypic effects in melanoma between receptor tyrosine kinases that confer resistance to BRAF inhibitors.** Frederic Zhentao Li, Rachel Ramsdale, Amardeep Dhillon, Grant McArthur, Petannel Ferrao. Peter MacCallum Cancer Center, Melbourne, Australia.

The majority of melanoma patients treated with a BRAF inhibitor (BRAFi) eventually acquire drug resistance. A major mechanism for acquired resistance is the activation of specific receptor tyrosine kinases (RTKs). We have recently shown that inherent and early adaptive resistance is associated with activation of the JNK-JUN pathway, decreased levels of the SPROUTY negative regulators and a mesenchymal-like phenotype. RTKs have been reported to be regulated by SPROUTY proteins and are able to mediate cell migration and invasion. In this study, we compared the expression and activation of specific RTKs previously linked to BRAFi resistance, within the same cellular background.

EGFR, FGFR1, MET and IGF1R were constitutively expressed in the BRAFi-sensitive melanoma cell line A375. The impact of ligand activation of each of these receptors in the presence of BRAFi on proliferation, survival, signalling, morphology, expression of phenotype markers and cell migration was assessed. We also overexpressed SPROUTY2 using an inducible system, to study its role in regulating RTK-mediated BRAFi resistance.

In A375 cells, overexpression and activation of either EGFR, FGFR1 or MET were able to reverse inhibition of cell proliferation induced by BRAFi, whereas IGF1R expression and activation was able to reduce cell death induced by high doses of BRAFi, but was unable to reverse inhibition of cell proliferation. The RTKs differed in their ability to activate signalling following treatment with BRAFi. In A375 cells, EGFR, FGFR1 and MET activated by their respective ligands, predominantly induced ERK signalling, whereas ligand activated IGF1R predominantly induced AKT signalling. EGFR activation conferred resistance to BRAFi by overcoming RAF-MEK-ERK pathway inhibition. Activation of EGFR was also able to prevent the early drug adaptive phenotype and reverse the BRAFi-induced changes in the expression of c-JUN, SNAI2, MITF and ZEB2. In contrast activation of IGF1R did not reverse the early drug adaptive phenotype induced by BRAFi. Combination treatment with either an IGF1R or AKT inhibitor was sufficient to overcome IGF1R-mediated survival. Enforced expression of SPROUTY2 reversed the resistance mediated by EGFR or FGFR1 activation. SPROUTY2 expression also conferred sensitivity to BRAFi in LOX-IMVI cells that were inherently resistant to BRAFi and expressed activated EGFR and FGFR1.

Together, our data suggests that RTKs vary in the signalling pathways that are induced upon ligand-activation, particularly in the context of BRAFi resistance. Our findings have clinical relevance for the rational design of drug combinations to target specific RTK signalling and overcome RTK-mediated resistance.
C72 Combined pan-RAF and MEK inhibition overcomes multiple resistance mechanisms to selective RAF inhibitors. Steven R. Whittaker1, Glenn S. Cowley2, Steve Wagner1, Flora Luo2, David E. Root2, Levi A. Garraway2. 1The Institute of Cancer Research, London, United Kingdom; 2The Broad Institute, Cambridge, MA.

Background: RAF and MEK inhibitors are efficacious in BRAF mutant melanoma but not in BRAF mutant colorectal cancer (CRC). To understand the underlying mechanisms of this difference we performed an RNA interference screen to identify loss of function events that sensitize CRC cells to RAF inhibition by PLX4720.

Methods: A pooled lentiviral library encoding 90,000 shRNAs, targeting 16,500 genes was transduced into the PLX4720-resistant, BRAF mutant RKO CRC cell line. The shRNA-infected cells were split into two experimental arms treated with either PLX4720 or DMSO. After 16 population doublings, the abundance of each shRNA was determined by PCR amplification and deep sequencing of the barcoded shRNA pool. The log-fold change in shRNA reads in the drug-treated condition was calculated relative to the vehicle control. RNAi gene enrichment (RIGER) was employed to rank genes that promoted sensitivity to PLX4720. Candidate genes were validated individually and their effects on cell proliferation, survival and MAPK pathway inhibition assessed.

Results: Genes involved in maintaining MAPK pathway activity scored prominently in our screen. Knockdown of MET, SHP2, SHOC2 and CRAF by shRNA sensitized cells to PLX4720. Efforts to suppress cell proliferation via single agent pan-RAF or MEK inhibition were more effective across melanoma and colorectal cancer cell lines than PLX4720. Strikingly, combined pan-RAF and MEK inhibition elicited a synergistic response in cell lines showing intrinsic resistance to PLX4720 associated with NF1 or KRAS mutation.

Conclusions: Resistance to BRAF inhibition is mediated by reactivation of the MAPK pathway in a CRAF-dependent manner. Pan-RAF inhibitors synergize with MEK inhibitors to suppress proliferation and induce apoptosis in both BRAF mutant, PLX4720-resistant cell lines and also in KRAS mutant cell lines. Pan-RAF inhibitors are progressing into clinical trials and our data support their use in combination with MEK inhibitors.

C73 A drug-modifier whole-genome shRNA screen identifies novel synthetic lethal interactions with PI3K inhibition in breast cancer. Yaara Zwang1, Casandra Chen2, The Broad Institute Genomic Perturbation Platform2, William C. Hahn1. 1Dana-Farber Cancer Institute, Boston, MA; 2The Broad Institute, Cambridge, MA.

The PI3K pathway is frequently activated in tumors, and was previously shown to be essential for tumorigenesis and tumor cell survival. Never the less, the main response to PI3K inhibition is cytostatic, therefore retaining a significant reservoir of tumor cells with a capability to re-grow and acquire resistance. Our goal is to identify genes that exhibit a synthetic lethal interaction with PI3K inhibition, namely, converting the response to PI3K inhibition from cytostatic to cytotoxic. To this end, we conducted a positive-selection whole-genome shRNA screen in a breast cancer cell line bearing a PI3K activating mutation, also exhibiting a robust cytostatic response to PI3K inhibition. Cells were infected with a pooled shRNA library consisting of 98k shRNAs, targeting ~17K genes. Following selection, cells were either left untreated, or treated with a PI3K inhibitor (GDC0941) for 2-5 days. Based on cleaved-PARP staining, apoptotic cells were sorted by flow cytometry, and sequenced to identify the shRNAs in the sorted population. We ranked shRNAs according to the significance of their relative occurrence between non-treated and treated samples, thereby identifying shRNAs that induce cell death only upon PI3K inhibition.
We validated potential hit genes using an arrayed viability assay, in which we included multiple shRNAs targeting each of the genes. Using seed-control shRNAs and rescue experiments, we confirmed on-target effects of the identified shRNAs, collectively targeting 5 genes. We further confirmed the relevance of the identified genes by testing the shRNAs in additional PI3K-activated breast cancer cell-lines, and in combination with additional PI3K-pathway inhibitors. Interestingly, the effect of the identified genes was specific to inhibition of the PI3K pathway, and was not observed upon unrelated growth inhibition.

All together, we identified five genes that exhibit robust synthetic lethal interaction with PI3K inhibition in breast cancer, of which 2 genes are protein kinases, PIM2 and ZAK, thus are potential co-targets with PI3K.

**C74** Establishment and characterization of a HER2-positive, TDM1-resistant PDX breast model, Michael J. Wick, Teresa L. Vaught, Justin Meade, Monica Farley, Armando Diaz, Anthony W. Tolcher, Drew W. Rasco, Amita Patnaik, Murali Beeram, Kyriakos P. Papadopoulos. START, San Antonio, TX; STOH, San Antonio, TX.

Background: Ado-trastuzumab emtansine (T-DM1), a recently approved antibody-drug conjugate (ADC), is approved for treatment of high HER2 expressing (3+), trastuzumab resistant breast and gastric cancers. While this agent is effective, resistance often develops. To better understand the mechanisms for resistance to TDM1 in high HER2 expressing breast cancer we collected tissue from a patient following response and progression to TDM1 and established a xenograft model for preclinical drug screening. The resulting model ST1360B was genomically characterized and screened in vivo using high dose TDM1; anti-tumor activity was compared with two trastuzumab-resistant but TDM-1 sensitive HER2 (3+) breast PDX models designated ST225 and ST340.

Methods: The high HER2 expressing (3+) TDM1 resistant breast PDX model ST1360B was established in athymic mice using pleural fluid collected from a thoracentesis. Establishment of the HER2 (3+) ST225 and ST340 models has been previously described. Clinical and PDX tissues were subjected to genomic analysis; for each model, DNA was extracted and subjected to comparative genomic hybridization and exon sequencing of known oncogenes; growth factor receptor and ligand densities was interrogated using immunohistochemistry. Drug sensitivity studies were performed evaluating sensitivity of models to single agent T-DM1, administered intravenously at 10 mg/kg on Day 0; weekly TDM1 dosing at 5 mg/kg was also evaluated in the ST1360B model Study endpoints included tumor volume and time from treatment initiation with tumor growth inhibition, delay and regression reported at study completion.

Results: Genomic analysis of ST1360B identified amplified HER2 and TOP2A; interestingly CD44 was found highly amplified, suggesting a possible mechanism for TDM1 resistance in this model. Mutations were not identified in PIK3CA or other relevant genes. In efficacy studies TDM1 was confirmed active towards ST225 and ST340 model including tumor regressions and durable complete responses in both models (TGI>100%). However TDM1 was not effective in ST1360B, with only transient tumor growth inhibition even with weekly drug administration.

Conclusion: We have established a model of TDM1 HER2 3+ resistant breast cancer and have characterized the model using comparative genomic hybridization and exon sequencing of known oncogenes, identifying amplified CD44 as a potential mechanism for drug resistance. This model can be utilized to better understand and to develop novel therapies to TDM1 resistance.
The purpose of our study was to define a method and mechanism for overcoming the resistance of clinically relevant KRAS-mutant/LKB1-deficient NSCLC cells to the BET bromodomain inhibitor JQ1.

LKB1 (Serine/threonine kinase 11) is mutated with loss of function in conjunction with mutated KRAS in 7-10% of NSCLC. Importantly, KRAS-mutant/LKB1-deficiency is associated with tumor aggressiveness and poor survival in human patients as well as in genetically engineered mouse models. Indeed, although the BET bromodomain inhibitor JQ1 dramatically reduces tumor volume in KRAS mutant mice, it has little effect in KRAS-mutant/LKB1-deficient mice. BET bromodomain proteins are chromatin readers that facilitate binding and activity of transcription factors such as the oncogene MYC. As such, JQ1 inhibits the transcriptional program of MYC and decreases MYC in mice.

To overcome JQ1 resistance in KRAS-mutant/LKB1-deficient NSCLC, we proposed to test JQ1 combination with chemotherapy, as such a combination would be a necessary arm of a clinical trial testing a BET bromodomain inhibitor. Here, we present data showing that gemcitabine permits and potentiates JQ1-mediated apoptosis of multiple KRAS-mutant/LKB1-deficient human NSCLC cell lines above either monotherapy. Increased apoptosis was observed by AnnexinV and TUNEL flow cytometry, PARP cleavage, and Comet assays.

Mechanistically, the drug combination had minimal effects on DNA damage and repair molecules in the KRAS-mutant/LKB1 deficient NSCLC cell line A549. Interestingly, however, JQ1 and combination with gemcitabine had profound effects on apoptotic molecules. JQ1 alone showed a robust increase in pro-apoptotic BimEL, BimL, and BimS that was balanced by a large increase in anti-apoptotic Mcl-1 24 hrs post-treatment. Similarly, MYC knockdown also increased Mcl-1, suggesting that JQ1 upregulation of Mcl-1 was at least partly dependent on JQ1 suppression of MYC. On the other hand, JQ1 combination with gemcitabine showed an increase in Bim that was not associated with increased Mcl-1.

Given these findings, we reasoned that KRAS-mutant/LKB1-deficient cells were resistant to JQ1-induced apoptosis executed by Bim because of protection provided by increased Mcl-1. In support of this hypothesis, Bim knockdown prevented JQ1-induced PARP cleavage in JQ1-sensitive H441 cells. Furthermore, Mcl-1 knockdown cells showed increased Bim, decreased Bcl-2, and increased PARP cleavage 48 hrs after JQ1 treatment in otherwise JQ1-resistant A549 cells.

Collectively, KRAS-mutant/LKB1-deficient cells are resistant to JQ1-induced apoptosis because of a compensatory increase in Mcl-1. This resistance can be overcome by combinatorial treatment with gemcitabine or direct Mcl-1 suppression, restoring apoptosis. Therefore, combinatorial treatment of a BET bromodomain inhibitor with gemcitabine or a Mcl-1 inhibitor may represent a potential novel strategy for treating the clinically relevant KRAS-mutant/LKB1-deficient subtype of NSCLC.
C76 A novel small molecule inhibitor of constitutive androstane receptor (CAR) that resensitizes doxorubicin-resistant neuroblastoma cells. Milu T. Cherian, Apana A. Takwi, Wenwei Lin, Taosheng Chen. St. Jude Children's Research Hospital, Memphis, TN.

This study aims to identify new specific small molecule inhibitors of the xenobiotic receptor CAR (constitutive androstane receptor) which could be used to increase sensitivity of resistant cancer cells to chemotherapeutics.

CAR and PXR are xenobiotic sensors that respond to drugs and endobiotics by modulating the expression of metabolic genes that enhance detoxification and elimination. Elevated levels of drug metabolizing enzymes and efflux transporters resulting from CAR activation promote the elimination of chemotherapeutic agents leading to reduced therapeutic effectiveness. Interestingly, all previously reported CAR inverse-agonists are also activators of PXR thus rendering them mechanistically counterproductive in tissues expressing both receptors. Using a directed screening approach, we discovered a potent small molecule, CINPA1, capable of reducing CAR-mediated transcription with an IC50 of ~70 nM, without activating PXR. CINPA1 is a specific xenobiotic inhibitor and has no general cytotoxic effects up to 25 \( \mu \)M. CINPA1 effectively inhibits CAR-mediated gene expression in all donors of human hepatocytes that express CAR endogenously. The mechanisms by which CINPA1 inhibits CAR function were extensively examined. We show that CINPA1 association with the CAR ligand binding domain results in increased corepressor interaction and reduced recruitment of coactivators. In addition, chromatin immunoprecipitation in the presence of CINPA1 illustrates reduced CAR binding to the promoter regions of target genes.

Multidrug resistance in tumors after chemotherapy can be associated with errant CAR activity, as in the case of neuroblastoma. Treatment of doxorubicin-resistant neuroblastoma cells with CINPA1 resulted in reduced CAR activity and subsequent decrease in drug-transporter levels. In doxorubicin-resistant UKF-NB3 (neuroblastoma) cells, CINPA1 treatment increased doxorubicin sensitivity and decreased cell growth. This suggests that CAR inhibitors used in combination with existing chemotherapeutics could potentially be utilized to attenuate multidrug resistance and resensitize chemo-resistant cancer cells.

C77 Oncogenic drivers reactivate kinase-inhibited focal adhesion kinase (FAK) through phosphorylation of effector residues. Sheila Figel, Felicia Lenzo, Timothy Marlowe, William Cance. Roswell Park Cancer Institute, Buffalo, NY.

Introduction: Focal Adhesion Kinase (FAK) is a critical signaling molecule showing elevated expression in nearly all tumor types. Phosphorylation of key tyrosine residues within FAK underlies its dual roles as both a kinase and a molecular scaffold that integrates signals from major oncogenes. Though FAK has emerged as a major drug target, inhibitors developed to date singularly target its kinase activity, leaving the scaffolding of oncogenic drivers intact. Our studies demonstrate that cells treated with FAK kinase inhibitors PF-573228, PF-562271, and defactinib retain phosphorylation of several key tyrosine residues downstream of oncogenic receptor tyrosine kinases (RTKs).

Methods: We examined the phosphorylation status of key FAK scaffolding sites Y861 and Y925 in RTK expressing normal and cancer cells treated with FAK kinase inhibitors PF-573228, PF-562271, and defactinib. As Src kinase is known to directly phosphorylate Y861 and Y925, we also tested phosphorylation of these sites by RTKs in Src/Yes/Fyn (SYF) MEFs, which lack the major Src family kinases.

Results: In cancer cells treated with FAK kinase inhibitors, multiple RTKs (HER2/3, PDGFR, and EGFR) activated FAK through the phosphorylation of effector tyrosines. Activation of HER2/3 in MDA-MB-453
breast cancer cells, PDGFR in U-87 glioblastoma cells, and EGFR in A549 lung cancer cells all stimulated Y861/Y925 phosphorylation in the presence of each of the three FAK kinase inhibitors. Moreover, Y861/Y925 phosphorylation occurred downstream of PDGFR and EGFR in Src-null SYF MEFs, demonstrating that RTK FAK targeting does not require Src. Finally, in vitro studies demonstrated that RTKs can directly phosphorylate these sites on FAK.

Conclusion: We have identified a novel mechanism through which FAK retains signaling activity in the presence of kinase inhibitors. Continued FAK signaling in tumor cells which overexpress HER2, EGFR or PDGFR may therefore be a mode of clinical resistance to FAK kinase inhibitors.

C80 Development of EGFR C797S mutation in serial liquid biopsy assessments in the clinical practice setting. Kathryn F. Mileham1, Qing Zhang1, Carol J. Farhangfar1, Daniel E. Haggstrom1, Stephen Fairclough2, Oliver A. Zili2, Daniel R. Carrizosa1, Richard B. Lanman2, Edward S. Kim1. Levine Cancer Institute, Charlotte, NC; 2Guardant Health, Inc., Redwood City, CA.

Introduction: EGFR T790M has emerged as an important biomarker to predict response to 3rd-generation tyrosine kinase inhibitors (TKI). Therapies such as AZD 9291 and Rociletinib demonstrated impressive results in the clinical setting in patients with non-small cell lung cancer (NSCLC). However just as this resistance...
biomarker (T790M) has developed as a result of treatment with prior EGFR TKIs, EGFR C797S mutation is an acquired resistance mechanism to EGFR T790M inhibitors. This mutation was recently reported for the first time in clinical practice utilizing tissue-biopsy based next generation sequencing (NGS) and in the clinical trial setting in cell-free circulating DNA (cfDNA). Here we report the first case of a patient with NSCLC and emergence of C797S mutation utilizing a cfDNA biopsy-free NGS panel in serial assessments in the clinical setting.

Methods: Patients with NSCLC were seen at Levine Cancer Institute and had serial blood draws to assess molecular aberrations in cfDNA over time for comparison with radiographic reviews. Blood samples were sent to Guardant360 for assessment by NGS with a targeted cfDNA NGS panel for 68 genes with complete exon sequencing for all 28 exons in EGFR, and other genes in the panel. Mutant allele fractions (MAF) are reported as % of mutant DNA molecules divided by total molecules (mostly leukocyte DNA-derived) overlapping the same mutated nucleotide base position.

Results: A 50 year old African-American female never-smoker with lung adenocarcinoma developed a tissue-biopsy confirmed EGFR T790M mutation after 12 months on erlotinib for EGFR exon 19 deletion. She had significant clinical and radiographic responses to a 3rd generation TKI started May 2014. cfDNA NGS tests in March and April 2015 showed no genomic alterations detectable. In late May 2015, she developed clinical symptoms of cough, dyspnea and fatigue but no definitive radiographic evidence of progression on scans June 2015. She received steroids and antibiotics and continued treatment with the 3rd generation TKI. However, corresponding cfDNA NGS testing revealed emergence of multiple low level mutations and the original deletion in EGFR (EGFR L747_P753 DelInsS, T790M, C797S, and A755G variant of uncertain significance) and TP53 (all <1.1%). Repeat scans in July 2015 demonstrated clear progression of disease and a fourth cfDNA NGS test showed a rise in all low level aberrations previously detected in cfDNA: EGFR A755G 4.2%, TP53 P278R 3.6%, EGFR T790M 3.1%, EGFR L747_P753 DelInsS 2.9%, EGFR C797S 1.4%. NGS indicated that there may be 2 clones present for EGFR C797S but confirmation is required as disease progresses.

Discussion: Complete exon sequencing of cfDNA upon clinical progression uncovered the simultaneous re-emergence of the original driver EGFR exon 19 del and resistance driver mutation EGFR T790M as well as the emergence of the next resistance driver mutation EGFR C797S. The latter mutation interferes with covalent binding of a 3rd generation TKI and under treatment pressure, the tumor appears to have simultaneously evolved two different EGFR C797S clones at low MAFs. cfDNA can be a dynamic measure of tumor response, easily obtained from a patient on any visit with potential for detection before macroscopic/radiographic evidence of progression, as demonstrated in this case with tumor evolution of the EGFR T790M and C797S alterations.

C81 Identification of novel synergistic targets for rational drug combinations with PI3 kinase inhibitors using siRNA synthetic lethality screening against GBM. Yong-Wan Kim. Yonsei, Incheon, Korea.

Several small molecules that inhibit the PI3 kinase (PI3K)-Akt signaling pathway are in clinical development. Although many of these molecules have been effective in preclinical models, it remains unclear whether this strategy alone will be sufficient to interrupt the molecular events initiated and maintained by signaling along the pathways because of the activation of other pathways that compensate for the inhibition of the targeted kinase. In this study, we performed a synthetic lethality screen to identify genes or pathways whose inactivation, in combination with the PI3K inhibitors PX-866 and NVPBEZ-235, might result in a lethal phenotype in glioblastoma multiforme (GBM) cells. We screened GBM cells (U87, U251, and T98G) with a
large-scale, short hairpin RNA library (GeneNet), which contains 43,800 small interfering RNA sequences targeting 8,500 well-characterized human genes. To decrease off-target effects, we selected overlapping genes among the 3 cell lines that synergized with PX-866 to induce cell death. To facilitate the identification of potential targets, we used a GSE4290 dataset and The Cancer Genome Atlas GBM dataset, identifying 15 target genes overexpressed in GBM tissues. We further analyzed the selected genes using Ingenuity Pathway Analysis software and showed that the 15 genes were closely related to cancer-promoting pathways, and a highly interconnected network of aberrations along the MYC, P38MAPK, and ERK signaling pathways were identified. Our findings suggest that inhibition of these pathways might increase tumor sensitivity to PX-866 and therefore represent a potential clinical therapeutic strategy.

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Background. Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase, and its mutation, overexpression and gene fusion have been associated with various cancers. EML4-ALK fusion has been confirmed to be an oncogenic driver for a small subset of the lung cancers (~4% of NSCLC), and becomes an excellent drug target that led to the development of the first effective therapy, crizotinib, a tyrosine kinase inhibitor for anaplastic lymphoma kinase, for this disease. However, like any other cancer therapy so far, the treatment always led to the development of resistance, rendering them ineffective in the end. Understanding the mechanisms causing these resistances can potentially facilitate overcoming the resistance and extend patients’ life. However, lack of experimental model hinders this understanding.

Method. We have recently established a large collection of patient derived xenografts (~350 PDXs), and we screened some of them for alk gene fusions. We then tested the positive model for sensitivity to crizotinib, and keep it under several rounds of crizotinib treatments to select resistant model. The induced resistant model was subjected to genomic analysis to identify the changes that might potentially be responsible for the resistance.

Results. We identified one model, NSCLC LU1656, containing EML4-ALK fusion with elevated ALK expression. It has also been shown to respond well to crizotinib in vivo. The continued treatment eventually led to the development of resistance to crizotinib (LU2445), a situation that might occur in patients in the clinic under the same treatment. We have performed transcriptome sequencing of the parental sensitive tumor (LU1656) and the selected resistant tumor (LU2445). So far, we found both models expressed similar high levels of expression of ALK, but no additional mutations in ALK gene. There are other alterations either genetically or epigenetically, some seemingly related to the ALK signaling pathways. However, their role in resistance still need to be confirmed.

Conclusions. Induced crizotinib resistance in ALK-fusion lung PDX can be useful to investigate crizotinib resistant mechanism and future drugs overcoming the resistance.

References


Epigenetic Targets


SMYD3 (Set and Mynd Domain containing 3) is a lysine methyltransferase overexpressed in several cancer types including breast, prostate, pancreatic, and lung, and this overexpression is associated with poor clinical prognosis. Genetic knockdown of SMYD3 by shRNA has been shown to decrease proliferation in a range of cancer cell lines suggesting that inhibition of SMYD3 may have therapeutic utility.

In this presentation we describe the discovery and optimization of a novel series of oxindole sulfonamides and sulfamides with SMYD3 inhibitory activity. One of these compounds, EPZ030456, has a SMYD3 biochemical IC50 of 4 nM and is active in cells with an IC50 of 48 nM in a trimethyl MAP3K2 (MEKK2) in-cell western (ICW) assay. The crystal structure of this compound was solved with SMYD3 and the nucleotide substrate, S-adenosylmethionine and shows the oxindole portion of the molecule extends into the SMYD3 lysine binding channel. EPZ030456 shows < 30% inhibition at a 10 uM screening concentration against 17 histone methyltransferase targets tested, including SMYD2.

Further optimization within the series resulted in EPZ031686 which has similar potency to EPZ030456 with a biochemical IC50 of 3 nM and an ICW IC50 of 36 nM and in addition exhibits good bioavailability following oral dosing in mice. Hence, EPZ031686 is a suitable tool to study the role of SMYD3 in cancer and other therapeutic areas, using both in vitro and in vivo models.

ZEN-3694 is an orally bioavailable small molecule discovered and developed from a BET bromodomain inhibitor discovery platform. In vitro, ZEN-3694 selectively binds to both bromodomains of the BET proteins, inhibiting the interaction of acetylated histone peptide with IC50 values in low nM range. ZEN-3694 inhibits proliferation of MV4-11 AML cells with an IC50 of 0.2 uM, and inhibits MYC mRNA expression with an IC50 of 0.16 uM.

ZEN-3694 has also demonstrated strong activity against many solid tumor and hematological cell lines with sub-uM IC50 values. In vitro synergy with Standard of Care (SOC) agents has been shown in a wide variety of malignancies including Breast, Prostate, Lung, Melanoma, AML, and DLBCL. Xenograft studies conducted with ZEN-3694 in AML, prostate and breast cancer models have demonstrated that it is efficacious at well-tolerated doses, modulating target gene expression and halting tumor growth in a dose-dependent manner.

In the AR positive VCAP prostate cancer cell line, ZEN-3694 inhibits proliferation synergistically with the AR antagonists enzalutamide and ARN-509. In an in vitro enzalutamide resistance model characterized by the up-regulation of the glucocorticoid receptor (GR), GR expression was inhibited by ZEN-3694 in a dose-dependent manner. Sensitivity to ZEN-3694 was unaltered, suggesting that it could be a valid therapeutic approach in patients developing resistance to AR antagonists through GR induction.

Robust PD modulation has been observed across multiple matrices for ZEN-3694 and will be explored further in the clinic. Promising target validation data, excellent pharmacological properties, and robust activity of ZEN-3694 across a variety of hematological malignancy and solid tumor settings support the clinical development of ZEN-3694 in various oncologic indications.

C87  EZH2 inhibition leads to decreased proliferation in SMARCA4-deleted ovarian cancer cell lines. Sarah K. Knutson, Allison E. Drew, Christopher Plescia, Robert A. Copeland, Jesse J. Smith, Heike Keilhack, Scott Ribich. Epizyme, Inc., Cambridge, MA.

Introduction: The H3K27 histone methyltransferase EZH2 is the catalytic component of the polycomb repressive complex 2 (PRC2), and is amplified, overexpressed, or mutated in multiple cancer types, supporting its function as an oncogene. In addition to genetic alterations in EZH2 itself, distal genetic changes in other proteins can lead to oncogenic dependency on EZH2 activity. For example, we have previously established that cell lines and xenografts deficient in INI1 (SNF5/SMARCB1), a core component of the SWItch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex, display profound sensitivity and durable regressions in the presence of the selective EZH2 inhibitor tazemetostat (EPZ-6438). Intriguingly, a complete response was observed in a patient with an INI1-negative rhabdoid tumor who participated in the tazemetostat Ph1 dose escalation study. This suggests that these tumors are addicted to dysregulated PRC2 activity, and confirms the previously proposed antagonistic relationship of SWI/SNF with PRC2, which is perturbed in INI1-deficient tumors. The loss of INI1 induces inappropriate SWI/SNF function, abrogating the repression of PRC2 activity, resulting in Polycomb target genes, such as those involved in differentiation and tumor suppression, to become aberrantly repressed. In addition to deletion of INI1, there are numerous reports describing genetic alterations in other SWI/SNF complex members. Given the oncogenic dependency of INI1-deficient tumors on PRC2 activity, we sought to investigate the sensitivity of
other SWI/SNF mutated cancer types to EZH2 inhibition. Specifically, we investigated the effects of EZH2 inhibition in ovarian cancers carrying somatic mutations in the SWI/SNF complex members ARID1A and SMARCA4.

Methods and results: A panel of ovarian cancer cell lines of different histologies was subjected to proliferation assays in 2-D tissue culture for 14 days in the presence of increasing concentrations of an EZH2 inhibitor. Selected cell lines were also tested in 3-D cultures, as it has been suggested in the literature that this context is necessary to observe anti-proliferative effects with EZH2 inhibitors. We found that ovarian cancer cell lines deficient in the SWI/SNF component SMARCA4 (also known as BRG1) are among the most sensitive in response to EZH2 inhibition, as demonstrated by decreased proliferation and/or morphology changes, at concentrations that are clinically achievable. In contrast, mutations in ARID1A, another SWI/SNF component, do not broadly confer sensitivity to EZH2 inhibition in ovarian cancer cell lines in either 2-D or 3-D in vitro assays. Furthermore, the effects of EZH2 inhibition on SMARCA4-negative ovarian cancer cells are context specific, since other cell types with SMARCA4 deletion, such as lung carcinoma cell lines, do not exhibit anti-proliferative affects with EZH2 inhibitor treatment.

Conclusions: These data suggest that tazemetostat may have therapeutic benefit in SMARCA4-deleted ovarian cancer, such as small cell cancer of the ovary of the hypercalcemic type (SCCOHT), which shows a high degree of loss of SMARCA4 expression. Further in vitro and in vivo studies are underway to interrogate these initial results further.

C88 HPV infections, hTERT methylation, and hTERT expression in patients with invasive cervical cancer. Pablo Moreno-Acosta1, Nicolas Morales1, Marcela Burgos1, Oscar Gamboa1, Juan Carlos Mejia1, Alfredo Romero-Rojas1, Alba Lucia Combita1, Monica Molano2. 1Instituto Nacional de Cancerología, Bogotá, D.C., Colombia; 2The Royal Women's Hospital, Melbourne, Australia.

Background: There is limited information of the role of hTERT methylation, hTERT expression and their association with type specific HPV infections in patients with invasive cervical cancer.

Objective: To analyze the possible association between methylation status of the hTERT promoter gene, hTERT expression and HPV infection in patients with invasive cervical cancer.

Methodology: Eighty seven frozen samples of women with invasive cervical cancer were analyzed for type specific HPV infection using a GP5+/GP6+ mediated PCR-RLB. hTERT DNA methylation analysis was performed on bisulfite modified DNA using a new PCR-RLB-hTERT methylation assay targeting two regions flanking the hTERT [region 1 (nt -240 to -1) and region 2 (nt +1 to +120) relative to first ATG]. Expression of hTERT was detected by immunohistochemistry using an Anti-hTERT (348-358) rabbit pAb).

Results: All samples with HPV types belonging to the α7 species (HPV 18, 45 and 59) showed no hTERT methylation in region 1 (core promoter) and an increase in % of partial methylation in region 2. Samples with HPV types belonging to the α9 specie (16, 52, 35, 31 and 58) showed similar % of methylation in region 1 and region 2 according viral type but the percentages of methylation were different between them (ranging from 0% for HPV58 samples to 66.7% for HPV31). Strong expression of hTERT was observed in 77% of the samples independent of the HPV genotype.

Conclusion: hTERT methylation seems to be associated with specific genotype HPV infection; however expression of the hTERT protein seems to depend of additional molecular events and worth further investigation.
C89  The small molecule inhibitor 4SC-202 controls aberrant HH signaling in cancer. Hella Kohlhof1, Wolfgang Gruber2, Daniel Vitt1, Fritz Aberger2, Tanja Prenzel1. 14SC AG, Martinsried, Germany; 2University of Salzburg, Salzburg, Austria.

Introduction: 4SC-202, a clinical stage inhibitor of LSD1 and HDAC1, 2 and 3 was investigated to identify its unique mechanism to target cancer cells by controlling aberrant Hedgehog signaling. Experiments: 4SC-202 is an oral available epigenetic modulator exhibiting a combined inhibition of the lysine specific demethylase LSD1 (KDMIA) and histone deacetylases HDAC1, 2 and 3. 4SC-202’s impact on Hedgehog signaling activity was tested in a SMO-dependent setting and in a SMO-independent setting. Therefore, Hedgehog signaling in DAOY cells was either activated by addition of sHH or Smoothened agonist or by stable depletion of SUFU for intrinsic, SMO-independent signaling. Pathway activity was determined by the expression of the effector protein GLI and primary target genes. SMO-independent Hedgehog signaling can be driven as well by growth factors like TGF-ß as demonstrated for the pancreatic cancer cell line PANC1. Therefore, 4SC-202’s impact on spheroid formation and protein expression was tested in PANC1 cells. Results: Compared to HDAC inhibitors like Vorinostat, LSD1 inhibitors like OGL-002 and Hedgehog inhibitors targeting SMO like Vismodegib, 4SC-202 was able to inhibit canonical as well as non-canonical GLI-driven Hedgehog signaling. Additionally, 4SC-202 was able to prevent spheroid formation of pancreatic cancer cells and inhibit TGF-ß driven signaling in PANC1 cells.

Conclusion: Aberrant activity of the Hedgehog signaling pathway has been implicated in the development, progression and relapse of different cancer entities. Especially the interaction of tumor cells and the microenvironment is driven by SMO-independent Hedgehog signals and therefore Hedgehog inhibitors targeting SMO like Vismodegib were not able to demonstrate clinical benefit in SMO-independent Hedgehog driven cancer types like PDAC. The unique feature of 4SC-202 to control both SMO-dependent and SMO-independent HH signaling provides the opportunity to demonstrate activity in Hedgehog driven entities in further clinical investigations. 4SC-202 was safe and well tolerated in a phase I clinical trial (TOPAS) in patients with advanced hematological diseases. With a disease control rate of 83%, one partial responder treated for 8 months and one complete responder treated for 28 months, patients benefited from 4SC-202 treatment and hints of activity could be demonstrated.

C91 Reversible lysine specific demethylase-1 inhibition is synergistic with docetaxel in castrate refractory prostate cancer. Sumati Gupta, Alexis Weston, Jared Bearss, Sunil Sharma. Huntsman Cancer Institute, Salt Lake City, UT.

Lysine specific demethylase-1 (LSD-1) expression correlates with poor survival in prostate cancer. LSD-1 is also key in androgen receptor signaling in androgen sensitive and castration refractory prostate cancer. LSD-1 is a chromatin modifying enzyme that in conjunction with its binding partners functions as an ‘erasor’ in the Epigenetic code process and demethylates lysine 4 and 9 residues on H3 protein resulting in gene repression or activation. Specifically in prostate cancer it demethylates H3k9me2 playing an important role in androgen receptor signaling promoting myc expression. Docetaxel chemotherapy is effective in prostate cancer but limited by toxicity and development of resistance. In this study we demonstrate synergy between LSD-1 inhibition and docetaxel in castrate refractory cell lines. We further demonstrate that LSD-1 inhibition is effective in docetaxel resistant cell lines. Cell survival assays were performed with HCI2509 on castrate refractory and docetaxel resistant prostate cancer cell lines. Histone methylation and myc expression were assessed. Colony formation assays, cell cycle analysis, xenograft studies were performed. Synergy studies were performed to assess the combination of 2509 and docetaxel. HCI2509 is cytotoxic to castrate refractory and docetaxel resistant prostate cancer cell lines in low micromolar doses. Treatment
with HCI2509 resulted in dose dependent increase in H3K4me2, H3K4me3, H3K9me2 levels and decrease in myc protein. RT-PCR showed a significant decrease in MYC expression upon treatment with HCI2509. Cell cycle progression was arrested at G0/G1 upon treatment with 2509 with inhibition of colony formation at 10 to 100 fold lower concentrations. PC3 xenografts in mice showed significant reduction in tumor burden upon treatment with 2509 with increased H3K9me2 marks and reduced myc protein levels. LSD-1 inhibition with HCI2509 has therapeutic potential in castrate refractory and docetaxel resistant prostate cancer. It inhibits myc expression and sensitizes castrate refractory prostate cancer to docetaxel.

C92 HCI-2577 inhibits LSD1 and modulates histone marks in Ewing's sarcoma models. Jared J. Bearss, Adrianne Neiss, Xiao-Hui Liu, Hariprasad Vankayalapati, Sunil Sharma. Huntsman Cancer Institute, University of Utah, Salt Lake City, UT.

Lysine-specific demethylase 1 (LSD1/AOF2/KDM1A) is a flavin-dependent histone demethylase that catalyzes the posttranslational oxidative demethylation of mono- and dimethylated lysines on histones. Methylation of lysine residues on histones can signal transcriptional activation or repression depending on the specific residue involved. H3K4me2 is a transcription-activating mark, and demethylation of this mark by LSD1 prevents expression of tumor suppressor genes important in human cancer. Whereas, H3K9 methylation is a repressive mark and LSD1 activity has been shown to upregulate tumor promoting pathways. This makes LSD1 emerge as an important target for the development of novel antitumor inhibitors. The compound HCI-2577 was identified as a potent reversible inhibitor of LSD1 enzymatic activity, with an IC50 of 7nM. In a diverse cell screen panel for cellular viability, Ewing's sarcoma was identified as being sensitive to HCI-2577. Here we show that HCI-2577 is efficacious in in vivo models of Ewing's sarcoma as a single agent with a favorable drug profile. In conclusion, HCI-2577 is a novel LSD-1 inhibitor with therapeutic potential in Ewing's sarcoma demonstrating promising activity in biochemical, cell-based and in vivo assays.

Gene Therapies

C93 RYBP predicts survival of patients with non-small cell lung cancer and sensitizes chemotherapy. wei wang1, Sukesh Voruganti1, Jiang-Jiang Qin1, Biyun Qian2, Ruiwen Zhang1.1Texas Tech University Health Sciences Center, Amarillo, TX; 2Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers and associates with a poor prognosis. Despite several targeted therapies have been developed with promising results, the therapeutic options for NSCLC are limited and the 5-year survival rate remains poor (~15%). Therefore, there is an urgent need for a better understanding of its pathogenesis and exploring novel therapeutic targets. Ring1 and YY1 binding protein (RYBP) is a member of polycomb group (PcG) proteins that typically act as transcriptional repressors via epigenetic modification of chromatin and participate in the establishment and maintenance of cell fates. Recent studies have shown that RYBP also possesses PcG-independent functions that promote apoptosis and cell cycle arrest in cancer cells. Our previous study has shown that RYBP stabilizes p53 through inhibiting MDM2 activity. Recent study indicates that RYBP is downregulated in human cancers, including NSCLC, but the underlying mechanisms are largely unknown. The present study was designed to demonstrate the molecular role of RYBP in NSCLC development and progression, chemotherapy, and patient survival. We systemically investigated the expression of RYBP in human NSCLC tissues and matched non-cancerous samples and evaluated the associations between the RYBP expression...
in NSCLCs and patient survival. We also generated a replication-deficient recombinant adenovirus driving the expression of RYBP (Adv-RYBP) and carried out in vitro and in vivo studies to explore the molecular basis for the tumor suppressing role of RYBP in NSCLC. We first demonstrated that the RYBP mRNA and protein expressions levels were significantly down-regulated in NSCLC tissues compared with matched noncancerous lung tissues. The low expression of RYBP was significantly associated with the poor prognosis in NSCLC patients. We then identified that enforced RYBP expression decreased cell viability, inhibited colony formation and induced apoptosis in NSCLC cells, while RYBP knockdown led to the opposite effects. RYBP affected the expression of several apoptosis-related proteins; and promoting apoptosis was one of the main mechanisms of RYBP-mediated cell growth inhibition in NSCLC. Moreover, AdRYBP led to a decreased NSCLC tumor xenograft growth. Additionally, clinically used chemotherapeutic agents induced the expression of RYBP, and RYBP sensitized NSCLC cells to chemotherapy in vitro and in vivo. In conclusion, our results reveal that RYBP is a potential biomarker for the diagnosis and prognosis of NSCLC and that reactivating RYBP in cancer cells may provide an effective and safe therapeutic approach to NSCLC therapy. (Supported by NIH/NCI grants R01 CA186662).

Hormonal Agents and Therapy

C94 Dual downregulation of Myc and AR with a sphingosine kinase-2 inhibitor prevents prostate cancer progression. Randy S. Schrecengost1, Staci Keller1, Matthew Schiewer2, Karen Knudsen3, Charles Smith1, 1Apogee Biotechnology Corp., Hummelstown, PA; 2Thomas Jefferson University, Philadelphia, PA.

The bioactive sphingolipid sphingosine-1-phosphate (SIP) drives several hallmark processes of cancer, making the enzymes that synthesize SIP, i.e. sphingosine kinase 1 and 2 (SK1 and SK2), important targets for cancer drug development. ABC294640 is a first-in-class SK2 inhibitor that effectively inhibits cancer cell growth in vitro and in vivo. Given that AR and Myc are two of the most widely implicated oncopgenes in prostate cancer (PCa), and that sphingolipids impact signaling by both proteins, the potential for using ABC294640 in the treatment of PCa was evaluated. This study demonstrates that ABC294640 abrogates signaling pathways requisite for PCa growth and proliferation. Key findings validate that ABC294640 treatment of early stage and advanced PCa models downregulate Myc and AR expression and activity. This corresponds with significant inhibition of growth, proliferation, and cell-cycle progression. Finally, oral administration of ABC294640 was found to dramatically impede xenograft tumor growth. Together, these results support the hypotheses that SK2 activity is required for PCa function and that ABC294640 represents a new pharmacological agent for treatment of early stage and aggressive PCa.

C95 ARV-330: An androgen receptor PROTAC degrader for prostate cancer. James D. Winkler1, Meizhong Jin1, Andy P. Crew1, AnnMarie K. Rossi1, Ryan R. Willard1, Hanqing Dong1, Kam Siu1, Jing Wang1, Deborah A. Gordon1, Xin Chen1, Caterina Ferraro1, Craig M. Crews2, Kevin Coleman1, Taavi K. Neklesa1. 1Arvinas, Inc., New Haven, CT; 2Yale University, New Haven, CT.

Patients with prostate cancer who progress on therapy often have enhanced Androgen Receptor (AR) signaling due to several mechanisms: increased androgen production, increased AR expression and/or specific AR mutations that render current therapies ineffective. A novel approach to block AR signaling is to specifically target AR for degradation. To do this, we have created AR PROTACs (PROtein-Targeting Chimeras), bi-functional molecules that have an AR binding moiety on one end and an E3 ligase-recruiting element on the other end, which leads to AR ubiquitination and degradation. We have applied this
technology to determine if it could address mechanisms of resistance to current therapy in prostate cancer models.

Our lead AR PROTAC, ARV-330, degrades AR in LNCaP and VCaP cells with 50% degradation concentrations (DC50s) < 1nM. AR degradation had functional consequences in cells, suppressing the AR target gene PSA, inhibiting proliferation, and potently inducing apoptosis in VCaP cells, with maximal apoptosis observed around 20 nM, versus 1 uM for enzalutamide. While both ARV-330 and enzalutamide block proliferation of VCaP cells in response to 0.1 nM of the AR agonist R1881, enzalutamide lost anti-proliferative potency with increasing R1881 concentrations, whereas ARV-330 maintained anti-proliferative effects. In cells containing the ARF876L mutation, enzalutamide was ineffective; however, ARV-330 maintained complete effectiveness. In mice, ARV-330 exhibited good pharmacokinetic properties, with t1/2 values of several hours and bioavailability of >80% after sc injection. Treatment of mice with ARV-330, at doses ranging from 0.3 to 10 mg/kg, resulted in reduction of AR protein levels and prostate involution in normal mice and, in mice implanted with VCaP tumors, reduction in plasma PSA and blockade of tumor growth.

In summary, the AR PROTAC ARV-330 removes AR from prostate cancer cells in a potent manner and produces therapeutic effects as a result. This cellular efficacy has translated into biomarker activity and efficacy in animal models, and ARV-330 is now in preclinical development. Thus, targeted degradation of AR may provide a novel mechanism for providing efficacious therapy for patients with prostate cancer for whom current therapies have failed.

C96 Thyroid hormone induced Beta-catenin-dependent proliferation in colorectal cancer cells. Hung-Yun Lin. Taipei Medical University, Taipei, Taiwan.

Thyroid hormone has been shown to induce proliferation of a variety of human cancer cell lines via a cell surface binding site on integrin αvβ3. Clinical studies indicate that thyroid hormone may support the growth of brain tumor, breast cancer, head-and-neck cancer, renal cell carcinoma and several other cancers. The effect of microenvironmental thyroid hormone on the growth of colorectal cancer has not been investigated fully. Numerous evidences suggest that the Wnt/β-catenin pathway, which is involved in both normal and oncogenic developments of the gut, is associated with thyroid hormone and its downstream signaling. The study is therefore aimed to look at the role of thyroid hormone, thyroxine (T4) on colon cancer progression through β-catenin activation.

Materials and Methods: The effect of T4 in two APC mutant colon cancer cell lines HT-29 and Colo205 cells and one APC wild type colon cancer HCT116 cells were studied. MTT assays were performed to evaluate cell proliferation under different concentrations of T4. Relative mRNA and protein expressions of relevant genes were examined using qPCR and Western blotting, respectively.

Results: T4 (10^-8 to 10^-6 M) induced proliferation in colorectal cancer HT-29 cells, HCT116 cells and Colo205 cells in a concentration-dependent manner. In HT29 and Colo205 cancer cell lines, the expressions of β-catenin and HMGA2 were not significantly altered by the application of T4. Alternatively, T4 increased accumulation of nuclear β-catenin and HMGA2 in HT-29 cells. Nuclear β-catenin and message were elevated by T4 in Colo205 cells. On the other hand, in addition to the cell proliferation and nuclear accumulation of β-catenin and HMGA2, T4 (10^-8-10^-6 M) induced the expression of β-catenin and HMGA2 in HCT116 cells. The expression of genes involved in proliferation was enhanced by thyroxine treatment.
Conclusions: T4 promoted proliferation in colon cancer HT-29, Colo205 and HCT116 cells. The effect of T4 in colon cancer involved nuclear distribution of β-catenin and its activation. However, the different phenotypes of APC affected the accumulation of nuclear β-catenin and HMGA2 induced by T4. Further study concerning detailed mechanisms is required to elucidate the role of thyroid hormone in colon cancer progression. By understanding the mechanisms behind the pathogenic effects of thyroid hormone, we will propose and develop new treatment approaches to colorectal cancers.

C97 Beyond intratumoural steroidogenesis: abiraterone resistance mediated by AR variants and glucocorticoid receptor signalling. Jan Matthijs Moll1, Johannes Hofland1, Wilma Teubel1, Corrina M.A. de Ridder1, Anne E. Taylor2, Ralph Graeser2, Wiebke Arlt2, Guido W. Jenster1, Wytske M. van Weerden1. 1Erasmus MC, Rotterdam, Netherlands; 2Centre for Endocrinology, Diabetes and Metabolism, University of Birmingham, Birmingham, United Kingdom; 3IMI-PREDECT, Brussels, Belgium.

Introduction: Castration resistant prostate cancer (CRPC) remains dependent on androgen receptor (AR) signalling, driven by adrenal precursors and potentially de novo steroid synthesis in other organ tissues including prostate. Abiraterone, an inhibitor of the steroidogenic enzyme CYP17A1 and the AR has been demonstrated to prolong survival of CRPC patients. In this study, we created a co-culture model using human prostate and adrenal tumours to study abiraterone resistance.

Materials and Methods: Human androgen-dependent PC (VCAp) and CRPC clones were cultured with substrates for de novo androgen synthesis or with adrenal androgens, or cultured with human adrenal cells (H295R) and treated with either the CYP17A1 inhibitor abiraterone or the antiandrogen MDV3100. Male mice bearing VCAp tumours and human adrenal H295R xenografts were castrated and treated with placebo or abiraterone. Tumour response was assessed by tumour growth, PSA release, steroid quantitation by (LC/MS-MS), immunohistochemistry and mRNA expression analysis of steroidogenic enzymes and nuclear receptors.

Results: In vitro, physiological levels of adrenal androgen precursors DHEA and androstenedione induced cell growth in parental and CRPC VCAp sub clones, whereas precursor steroids pregnenolone and progesterone for de novo synthesis did not. In a co-culture model, abiraterone blocked H295R-induced growth of VCAp cells. Likewise, in vivo, H295R tumours stimulated castration-resistant VCAp growth. This stimulative effect was inhibited by abiraterone, reducing but not fully blocking growth and PSA production. In the absence of H295R tissue, VCAp xenografts grew slow but became castration resistant nonetheless. In contrast to the observed effects on VCAp growing in castrate animals bearing H295R tumours, abiraterone was unable to inhibit the slow VCAp growth and low PSA production in castrate mice without H295R xenografts. LC/MS-MS analysis of plasma and tumour tissue could not confirm increased de novo production of androgens. Castrate and abiraterone-resistant VCAp tumours were characterised by increased levels of AR, AR variants and glucocorticoid receptor (GR) expression, resulting in equal AR target gene expression levels.

Conclusions: Our data indicate that AR ligand dependent regrowth of CRPC is predominantly supported via adrenal steroid production. Abiraterone resistant disease of VCAp relies on AR overexpression, expression of ligand independent AR variants and GR signalling.
C98  Selective estrogen mimics (SEMs) for the treatment of tamoxifen resistant breast cancer. Hitisha Patel, Rui Xiong, Lauren Gutgesell, Jiong Zhao, Mary Ellen Molloy, Debra Tonetti, Gregory R.J Thatcher. University of Illinois, Chicago, Chicago, IL.

Tamoxifen, an antagonist at estrogen receptor alpha (ERα) in breast tissue, and the prototypical selective estrogen receptor modulator (SERM), is the standard of care for many patients with ER-positive breast cancer. However, continued tamoxifen treatment increases the risk of endometrial cancer and half of ER-positive breast cancer patients do not respond or relapse on treatment with tamoxifen. Tamoxifen-resistant tumors are often ER-positive and endocrine-independent and therefore resistant to aromatase inhibitors. Recent clinical trials have shown the efficacy of estrogen in patients who have undergone exhaustive tamoxifen therapy. Therefore, we propose the development of small molecules that mimic the actions of estradiol in ER-positive breast cancer without the uterotrophic actions of estradiol and tamoxifen: Selective Estrogen Mimics (SEMs). Evidence suggest that a significant subset of ER-positive breast cancer, overexpressing PKCα, and tamoxifen-resistant cancers will respond to SEMs. In order to design and optimize SEMs, tamoxifen-resistant cell lines, MCF-7:5C, T47D: PKCα and T47D: Tam1 were studied. The activation of classical ERα signaling by the estrogen mimics was profiled in MCF-7 cells and cell viability in 2D culture was examined. In an effort to increase throughput and mimic the in vivo environment more closely, we assessed the SEMs in a new 3D spheroid model. It was found that in MCF-7:5C cells in 2D and T47D spheroids that SEM induced cell death was mediated by activation of ERα. SEMs caused decreased spheroid viability and growth in all three models of tamoxifen resistance. Extranuclear localization of ER after SEM treatment previously reported in matrigel assays and in vivo xenografts was also observed in spheroids. This emphasizes the capability of the 3D spheroids to replicate in vivo environments, making them a high-throughput translational assay of choice for drug discovery. Three promising SEMs were evaluated in xenograft models of TAM-resistant, PKCα overexpressing breast cancer. While E2 caused regression of these tumors, a significant increase in uterine weight as predicted was observed. More importantly, SEM treated mice had negligible increase in uterine weight underlining the enhanced safety of these molecules. These data suggest that development of SEMs that retain the beneficial properties of estrogen while limiting the side effects is a feasible strategy for the treatment of tamoxifen resistant breast cancer.


Background: Inhibitors of androgen receptor (AR) signaling axis, such as enzalutamide and abiraterone, represent important advances in the management of castration-resistant prostate cancer (CRPC). However, resistance to these drugs is still occurring. Recent reports suggest that there are several mechanisms of resistance. Ligand independent AR activation such as induction of AR splice variants or c-Myc expression is a major emerging mechanism of CRPC progression. There is an urgent need for developing a novel therapy to address these unresolved issues.

Methods: For assay of androgen-independent AR transactivation, prostate cancer cells were transiently transfected with androgen-responsive reporter gene construct. The transfected cells were treated with growth factor and cytokine in steroid-depleted media, and luciferase activity was measured. To evaluate the effect of TAS3681 on AR and c-Myc protein expression, prostate cancer cells were treated with TAS3681 in steroid-depleted media. AR and c-Myc protein levels were determined by Western blot. Real-time PCR was
used to analyze the mRNA levels of c-Myc and c-Myc target gene. Chromatin immunoprecipitation was performed to determine the enrichment of AR at the element.

Results: TAS3681 dose-dependently reduced AR protein levels in prostate cancer cells. In contrast to enzalutamide, TAS3681 suppressed androgen-independent AR transactivation by growth factor and cytokine. In prostate cancer cells which express full-length AR and splice variant AR-v7, TAS3681 suppressed AR-v7 target gene expression through downregulation of AR-v7 occupancy at the enhancer. Moreover, TAS3681 reduced expression of c-Myc, critical driver of androgen-independent mechanisms of prostate cancer progression, via AR downregulation activity. In addition, real-time PCR assay showed the suppression of c-Myc and c-Myc target gene mRNA levels by TAS3681 but not by enzalutamide.

Conclusion: Our findings suggest that TAS3681, a new type AR antagonist with AR downregulation activity, has a potential to overcome ligand-independent AR activation and could be a candidate of breakthrough therapy for resistance to current AR pathway target drugs.

C100  RAD1901, an orally available selective estrogen receptor downregulator, has potent anti-tumor activity in in vitro and in vivo models of ER+ breast cancer. Teeru Bihani, Jeffrey Brown, Gary Hattersley, Fiona Garner. Radius Health, Waltham, MA; Pharmagellan, Milton, MA.

Breast cancer is the most frequent type of cancer diagnosed in women, with over 200,000 new cases diagnosed in the US each year. Treatment strategies are typically based on the tumor’s receptor status; that is, whether a tumor expresses estrogen receptor (ER), progesterone receptor (PR), or Her2. Estrogen receptor positive (ER+) breast cancers comprise approximately two-thirds of all breast cancers. For decades the approach to treat ER+ disease revolved around altering the ligand interactions with the receptor; either by preventing estrogen binding (e.g. tamoxifen) or preventing estrogen biosynthesis (aromatase inhibitors). While patients typically respond well to these agents, estrogen-independent ER activity and recurrent ER mutations, are increasingly being reported as contributing factors to endocrine resistance and continue to be a clinical hurdle. Given this growing unmet medical need, selective estrogen receptor downregulators, or SERDs, have gained widespread attention as new therapeutic treatment strategies for ER + disease. Indeed, fulvestrant has been shown to downregulate ER and cause tumor growth inhibition in many ER+ breast cancer models. However, in the clinic fulvestrant appears to be limited by PK exposure properties and this, combined with its intramuscular route of administration underscore the need for novel orally available SERDs. Here, we describe RAD1901, an orally administered SERD that binds ER and targets it for degradation in a dose-dependent manner. Biochemical affinity binding studies and cocrystallization experiments revealed insights into RAD1901 complexes with both wild-type and mutant forms of ER. In addition, RAD1901 treatment resulted in decreased cell proliferation in in vitro breast cancer cell lines and had profound single agent tumor growth inhibition in in vivo xenograft models. Consistent with these findings, RAD1901 treatment resulted in decreased expression levels of ER target genes. Interestingly, the extent of tumor growth inhibition induced by RAD1901 in vivo was dependent on ER expression levels, demonstrating the specificity of RAD1901 and predicting its activity in ER-driven cancers. Importantly, RAD1901 was also able to induce significant tumor growth inhibition in clinically relevant and representative patient-derived xenograft models, at a level similar to or greater than fulvestrant. In conclusion, our preclinical data demonstrate that RAD1901 is an orally available SERD, with potent single agent anti-tumor activity. RAD1901 is currently under clinical investigation in post-menopausal women with advanced ER+ disease.
Immune Mechanisms Invoked by Radiation Therapy

C101 Circulating tumor necrosis factor receptor (TNFR) proteins in systemic disease outcome to combined-modality radiotherapy in rectal cancer. Sebastian Meltzer¹, Erta Kalanxhi¹, Helga Helseth Hektoen², Svein Dueland³, Kjersti Flatmark³, Kathrine Røe Redalen¹, Anne Hansen Ree¹. ¹Akershus University Hospital, Lorenskog, Norway; ²University of Oslo, Oslo, Norway; ³Oslo University Hospital, Oslo, Norway.

Introduction: In colorectal cancer, the impact of the tumor microenvironment with its immune effectors for disease outcome is increasingly acknowledged. Following curatively intended combined-modality radiotherapy in locally advanced rectal cancer (LARC), metastatic disease still remains a dominant cause of failure. Therefore, within the frame of a prospective LARC study of intensified neoadjuvant therapy (NCT00278694), and in light of intriguing recent findings of radiation effects on systemic antitumor immunity, we investigated whether treatment toxicity and survival outcome might be reflected by circulating immune factors.

Experimental procedures: Using antibody array technology, the profiling of approximately 500 proteins was performed in serial serum samples collected during neoadjuvant chemotherapy (NACT; two cycles of the Nordic FLOX regimen) followed by long-course chemoradiotherapy (CRT) before final pelvic surgery. Array data was analyzed in the Significance Analysis of Microarrays algorithm, and functional associations among proteins were explored using the Search Tool for the Retrieval of Interacting Genes and Proteins software. Clinical endpoints were progression-free survival (PFS; median follow-up of 59 months for 85 patients) and treatment toxicities as assessed by Common Terminology Criteria for Adverse Events (CTCAE) scoring.

Results: Analysis of the array data revealed significant changes in levels (relative to baseline) of a number of serum proteins during the neoadjuvant treatment course, among which osteoprotegerin (OPG; TNFRSF11B) showed the strongest increase. Single-parameter immunoassay OPG assessment demonstrated significant correlations between its baseline serum levels (range 13.6-177 pg/ml) and patients’ age as well as common markers of systemic inflammation, such as erythrocyte sedimentation rate (ESR) and neutrophil-to-lymphocyte ratio (NLR). In univariate Cox regression analysis, high values of these variables were associated with adverse PFS (age-adjusted baseline OPG levels: hazard ratio (HR) 3.33, 95% confidence interval (CI) 1.24-8.94, p=0.02; baseline ESR: HR 1.85, 95% CI 1.10-3.11, p=0.02; post-NACT NLR: HR 1.89, 95% CI 1.08-3.31, p=0.03). Of further note, patients’ serum levels of RANK (TNFRSF11A) paralleled OPG alterations during the neoadjuvant therapy. Specifically, the CRT-induced changes in OPG and RANK were associated with PFS — the higher the increase (fold-change from NACT completion), the lower risk of a PFS event following definitive surgery (OPG: HR 0.27, 95% CI 0.09-0.87, p=0.03; RANK: HR 0.13, 95% CI 0.02-0.76, p=0.02). Correspondingly, by one-way analysis of variance, decline in serum levels during CRT (fold-change from NACT completion) was associated with adverse CTCAE grade diarrhea, the clinical correlate of treatment toxicity (OPG: p<0.01; RANK: p<0.01).

Conclusions: In this LARC study, elevated levels of systemic inflammation markers on commencement of neoadjuvant therapy were associated with adverse PFS. On the contrary, the association between strong increase in circulating TNFR proteins at CRT completion and favorable outcome in a patient population treated with curative intent but with metastatic disease as the main PFS event suggests that systemic antitumor immunity may be invoked by radiation.
C102 Combination of immune checkpoints DNA vaccines and radiation enhances melanoma control. Keng-Hsueh Lan\textsuperscript{1}, Ming-Feng Wei\textsuperscript{1}, Keng-Li Lan\textsuperscript{2}, Ann-Lii Cheng\textsuperscript{1}, Sung-Hsin Kuo\textsuperscript{1}. \textsuperscript{1}National Taiwan University Hospital, Taipei, Taiwan; \textsuperscript{2}National Yang-Ming University, Taipei, Taiwan.

Background: We intend to develop DNA-based vaccines targeting immune checkpoint proteins in order to enhance immune response to tumors. Ionizing radiation, similar to some conventional chemotherapy, possesses immunomodulatory properties. Therefore, we reason that in combination with radiation, which may expose tumor-related antigens to the T cells, these immune-modulating DNA vaccines may reverse tumor-induced immune evasion and create a sustainable anti-tumor immunity.

Materials and methods: The DNA sequences encoding murine CTLA-4 or PD1 were PCR amplified using a cDNA library obtained respectively from leukocyte of mouse and human as template. The resulting PCR products were fused with a transmembrane domain sequence of placental alkaline phosphatase (PLAP) into a mammalian expression plasmid, pVAC-1, forming CTLA-4 and PD1 DNA vaccines. These c57BL/6 mice (5 mice/group) were injected with DNA vaccine or control plasmid intramuscularly with the aid of electroporation weekly for 3 times. One week after the last vaccination, sera from the immunized mice was subjected to ELISA assay for detection of antibody titers against the respective immune checkpoint protein.

B16F10 murine melanoma tumors were established on the c57BL/6 mice. The radiation regimen used (when applicable) was 2 weekly fractions of 5 Gy after the tumors were established. The mice were divided into groups, each treated with either radiation, radiation plus CTLA-4 vaccine, radiation plus PD1 vaccine, radiation plus CTLA-4 and PD1 vaccines, or no treatment (control group).

Results: The mice vaccinated with the CTLA-4 or PD1 DNA vaccines demonstrated increased antibody titers against respective proteins, compared with the control groups (p < 0.005). Compared with radiation alone, the addition of the CTLA-4 vaccine led to enhanced tumor regression (a 53.2\% reduction of tumor volume relative to that of radiation alone group) 1 month after radiation started. However, immunization with PD1 vaccine did not significantly enhance the tumor suppression of radiation (a 14.7\% reduction of tumor volume; p=0.55). Interestingly, near total regression of B16 tumor was observed when the mice received radiation plus immunization with both CTLA-4 and PD1 vaccines, a 92.4\% decrease of tumor volume relative to that of radiation alone group (p = 0.037).

Conclusion: We have developed potent DNA vaccines targeting CTLA-4 and PD1 immune checkpoints, enhancing the tumor control effects of radiation in the melanoma animal model. These DNA-based vaccines have the advantages of being less expensive and easier to manipulate as opposed to the antibody-based reagents. The blockade of PD1 checkpoint alone is futile in aiding tumor control of radiation in the c57BL/6 melanoma model. The interplay of various aspects of immunity seems crucial in the effort to recruit its anti-tumor ability.

C103 The combination of PI3k\(\delta\) selective inhibition and immunomodulation shows efficacy in solid tumor models. Holly K. Koblish, Liang-Chuan Wang, Michael Hansbury, Yue Zhang, Gengjie Yang, Timothy Burn, Paul Waeltz, Mark Rupar, Eddy Yue, Brent Douty, Thomas Maduskuie, Nikoo Falahatpisheh, Yun-long Li, Andrew Combs, Gregory Hollis, Reid Huber, Peggy Scherle. Incyte Corporation, Wilmington, DE.

Understanding the in vivo responses to immunoregulatory agents provides a basis for building more efficacious combination regimens. Pharmacologic inhibition of the oncogenic PI3K\(\delta\) pathway has been shown to be active in patients with hematopoietic malignancies. Recently, genetic inactivation of PI3K\(\delta\) in
mice was shown to delay the growth of solid tumors, through the inactivation of Treg-mediated suppression of cytotoxic CD8+ T cell responses, suggesting that it may have additional utility in this patient population. We identified a similar immunomodulatory role for the PI3Kδ-selective inhibitor INCB050465 in a preclinical model of pancreatic cancer, where an increase in the number of CD8+ T cells, a decrease in the number of suppressor cells and efficacy were seen. Therefore we explored the potential of INCB050465 in additional preclinical solid tumor models, alone and in combination with other immunotherapeutic agents. INCB050465 inhibited tumor growth in multiple established tumor models which are not dependent upon oncogenic PI3K signaling. Tumor growth inhibition was not observed in these models in immunocompromised mice, demonstrating that the anti-tumor effects of these agents require an intact immune system. To further investigate immune-mediated mechanisms, tumors were analyzed for modulation of gene expression and immune phenotype after mice received short-term treatment. INCB050465 was shown to significantly downregulate the T cell gene signature in tumors, and this was primarily due to depletion of CD4+CD25+FoxP3+ regulatory T cells. As seen previously, the number of CD8+ T cells was shown to be higher in INCB050465-treated tumors. The combination of PI3Kδ and JAK inhibition resulted in enhanced activity in a T-cell-inflamed model by reducing both Treg and M2 macrophages, which promotes re-activation of both CD4+ and CD8+ T cells. In addition, PI3Kδ inhibition and PD-L1 blockade resulted in enhanced efficacy by depleting Treg and prolonging T cell response over time. In summary, pharmacological inhibition of PI3Kδ can enhance anti-tumor immunity by depleting Treg while increasing the numbers of cytotoxic CD8+ T cells. These data support clinical evaluation of the mechanism, and further studies to understand the molecular basis of efficacy and complex cellular responses may provide rationale to identify individuals who may benefit from PI3Kδ inhibitor-based immunotherapy combinations in the clinic.

C104 TAS-121, a highly potent and mutant-specific EGFR inhibitor, modulates the immune system, resulting in anti-tumor immune responses. Satoshi Fukaya, Yoshimi Aoyagi, Masanori Katoh, Kimihiro Itoh, Toshihiro Shibutani, Nozomu Tanaka, Tomonori Haruma, Akihiro Hashimoto, Kazutaka Miyadera, Kenichi Matsuo, Yoshikazu Iwasawa, Teruhiro Utsugi, Kazuhiko Yonekura. TAIHO PHARMACEUTICAL CO., LTD., Tsukuba, Japan.

Background: TAS-121 is one of the 3rd generation EGFR TKI, which specifically inhibits mutant EGFRs, and it leads to high cellular selectivity and potent antitumor activity in various in vivo models. TAS-121 is being investigated in patients with non-small cell lung cancer (NSCLC) harboring not only active mutant EGFR, but also acquired resistance mutation T790M in phase I study. Immune-related events observed during treatment with TAS-121 in preclinical and clinical studies suggested that TAS-121 could modulate the immune system and, consequently, might partially contribute to anti-tumor effect. To prove this hypothesis, we examined the immuno-modulating potential of TAS-121 using MLR assay, TCR stimulation assay and in vivo syngeneic tumor models.

Material and methods: Immune cell proliferation and cytokine production were confirmed by the CFSE dilution using flow cytometry, and ELISA assay, respectively. Anti-tumor immune response was analyzed in syngeneic models bearing with MC38 murine colon carcinoma cell line.

Results: TAS-121 strongly induced the proliferation of CD4 T cells and CD8 T cells as well as stimulating cytokine production. Other EGFR TKIs, including 1st generation to 3rd generation agents, didn't show these effects. TAS-121 inhibited the growth of syngeneic tumor, MC38 in vivo, while no growth inhibition was observed in vitro. These data demonstrate that TAS-121 can induce anti-tumor immune responses.
Conclusions: These findings suggest that TAS-121 could specifically modulate the immune system including T cells, leading to the anti-tumor immune responses. Therefore, TAS-121 has a unique profile among the 3rd generation EGFR-TKI. Considering this immune-modulating profile, combination with immune checkpoint blockade agent such as anti-PD1 monoclonal antibody might be beneficial for NSCLC patients harboring mutant EGFR.

C105 DNA damage response deficiency (DDRD) in breast cancer is associated with a STING-dependent innate immune response. Eileen E. Parkes1, Steven M. Walker1, Nuala McCabe1, Laura E. Taggart1, Laura Hill2, Karen D. McCloskey1, Niamh E. Buckley1, Manuel Salto-Tellez1, Paul B. Mullan1, D. P. Harkin1, Richard D. Kennedy1. 1Queens University Belfast, Belfast, United Kingdom; 2Almac Diagnostics, Craigavon, United Kingdom.

Background: In breast cancer the presence of an immune infiltration has been recognised as a prognostic factor, however the mechanisms underpinning this response are not clearly defined. Previously we identified a gene expression signature that defines a DNA damage response defective (DDRD) molecular subgroup in breast cancer, that is enriched for BRCA1 and 2 mutations, and has a good outcome following DNA-damaging chemotherapy. Importantly, this DDRD subgroup is associated with up-regulation of type I Interferon-related genes. We therefore investigated the mechanism activating this immune response in the context of abnormal DNA repair.

Results: IHC analysis demonstrated that both intra-tumoral and stromal CD8+ and CD4+ T cell infiltration were associated with DDRD positive breast tumors. The CXCL10 and CCL5 cytokines were shown to be significantly up-regulated in DDRD positive breast tumours and in tissue culture models for the DDRD molecular subgroup. Furthermore, conditioned media from DDRD positive cell lines stimulated inward migration of peripheral blood mononuclear cells, when compared to media from DDRD negative cells, indicating the presence of active cytokines. We identified constitutive activation of the innate immune pathway STING/TBK1/IRF3 specifically in DDRD positive cells when compared to DDRD negative cells and found that binding of the DNA sensor cGAS to cytosolic Histone H3 was required for this immune response. Treatment of HeLa cells with DNA damaging chemotherapies but not taxanes resulted in STING-dependent expression of CXCL10 and CCL5 cytokines.

Conclusion: We have identified that the STING/TBK1/IRF3 immune pathway is activated by endogenous DNA damage in DDRD breast cancers and may explain lymphocytic infiltration observed in this subtype of breast tumours. These data suggest that the DDRD signature can identify an innate immune response to DNA damage in breast cancer, which may have therapeutic applications for immune targeted therapies.

C106 CBP501 potentiates the appearance of cisplatin-induced indicators of immunogenic cell death and promotes anti-tumor effects in an immuno-competent mouse model. Keiichi Sakakibara1, Takuji Sato1, Donald W. Kufe2, Daniel D. Von Hoff3, Takumi Kawabe1. 1Canbas Co., Ltd., Numadu, Japan; 2Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA; 3Translational Genomics Research Institute (TGen), Phoenix, AZ.

Introduction: The CBP501 calmodulin-binding peptide is an anti-cancer drug candidate that has completed two Phase II clinical trials for patients with malignant pleural mesothelioma and non-small cell lung carcinoma (NSCLC). CBP501 was previously described as a unique G2 checkpoint-directed agent and as an enhancer of cisplatin (CDDP) uptake. In a post-trial analysis of patients with NSCLC, it was found that overall
survival (OS) was prolonged in a subpopulation of patients with normal white blood cell counts (WBC) (WBC < 8000). Here we show that modulation of immunogenic cell death might be a novel function of CBP501. Based on this novel activity of CBP501, combined treatment with PD-1 blocking antibodies in vivo was also explored.

Methods: CT26WT, a strain of CBP501 sensitive cells, were treated with clinically achievable dose levels of CDDP (10 or 20 uM) and 0.5 uM CBP501 for 0.75 h in vitro. After exchange with fresh drug-free medium, cells were incubated for 24, 48, or 72 h for IB analysis of phospho-eIF2-alpha, for calreticulin analysis by FACS, or for HMGB1 ELISA. The in vivo mouse study examined the effects of 3 dosing cycles for 3 anti-cancer agents, alone or in different combinations [CDDP: 5 mg/kg x 1/week, CBP501: 7.5 mg/kg x 3/week, anti-mPD1 antibody (RMP1-14): 200 ug x 1/week] using immuno-competent BALB/c mice bearing subcutaneous inoculated CT26 WT cells.

Results: In vitro, CDDP in combination with CBP501 elicited increased death in CT26WT cells, as well as an increase in different indicators of immunogenic cell death. These indicators include induction of phospho-eIF2-alpha, increase in cell surface-exposed calreticulin, and extracellular release of HMGB1. In an in vivo BALB/c mouse model, CDDP-treated mice showed a reduction of tumor growth by 52.7% as compared to vehicle-treated mice. CBP501 + CDDP showed an additional reduction of tumor growth by 63.1% as compared to vehicle-treated mice. Treatment with anti-mPD-1 antibody alone showed a slight reduction of tumor growth by 25.2% as compared to vehicle-treated mice. However, combined treatment with anti-mPD-1 + CDDP or anti-mPD-1 + CDDP + CBP501 showed significant reductions in the tumor growth in comparison to the vehicle-treated mice by 69.3% and 78.7%, respectively.

Conclusion: These results suggest that the anti-tumor activity of combined CBP501 + CDDP treatment is potentiated by inducing immunogenic cell death. This novel effect might contribute to the prolonged OS found in the phase II clinical trials. Combined treatments that include the anti-mPD-1 immune-checkpoint inhibitor were effective and shall be examined further. Further, immunohistochemical analyses of the effects of this combined treatment on tumor microenvironment are under way.

C107 Preclinical response of murine syngeneic tumor models to immune checkpoint inhibitor antibodies. Maryland Franklin, Matt Thayer, Chris Elders, Dan Saims, Scott Wise. Molecular Imaging, Ann Arbor, MI.

Tumor cell viability and proliferation is regulated by the balance between tumor self-survival and host elimination mechanisms. There are multiple mechanisms whereby tumors successfully evade host immune cell surveillance, permitting continued growth and proliferation. Immunotherapies are aimed at bolstering the host immune system. Delivery of these agents has been shown to actively eliminate disease in a number of patients to date. We evaluated several syngeneic mouse tumor lines for anti-tumor activity with anti-CTLA-4 and/or anti-PD-L1 antibodies, as well as the immunological changes occurring following treatment with these checkpoint inhibitors. The effectiveness of these inhibitors was assessed in the B16.F10 (melanoma), RIF-1 (sarcoma), 4T1-luc (mammary) and CT26 (colon) murine tumor models. Cells or tumor fragments (RIF-1) were implanted SC in the axilla. The anti-CTLA-4 9D9 antibody clone from Bio X Cell (West Lebanon, NH) was employed for responsiveness in each model. All treatments were initiated 3 days post implantation, prior to the appearance of established tumor, at inoculums known to have 100% take rates. The CT26 model was highly responsive with all animals showing tumor regressions and 70% tumor-free survivors at study end. The RIF-1 model was moderately responsive with several mice showing long term progression-free survival and a 56% increase in lifespan vs. the isotype control group. Both the B16.F10...
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and the 4T1-luc models were non-responsive. Tumor responsiveness to the anti-PD-L1 10F.9G2 antibody clone (Bio X Cell) was also compared in the RIF-1 and CT26 models. In contrast to the anti-CTLA-4 antibody studies, RIF-1 was found to be non-responsive and CT26 was moderately responsive to the anti-PD-L1 antibody. Evaluating immunological endpoints by flow cytometry is critical when assessing the effectiveness of immune checkpoint inhibitors. To this end, the immune profiles of both responsive (CT26) and non-responsive (4T1 and B16.F10) models were examined in the absence or presence of antibody treatments. Initial studies were focused on analysis of T cells (CD4 and CD8) as well as T regulatory cells (Tregs; CD4/CD25/FoxP3) and myeloid derived suppressive cells (MDSC; CD11b/Gr1). The 4T1 model was found to be highly MDSC driven and would be suitable to evaluate agents that target myeloid-derived cell populations. While this model was not very responsive to anti-CTLA-4 treatment, a trend toward decreased MDSCs following treatment was observed with anti-CTLA-4 and not the isotype control antibody. In contrast, the CT26 model was more highly mixed and contained populations of both Tregs and MDSCs. Treatment with anti-CTLA-4 increased the CD8+ T cell population and decreased the Treg cell population with minimal effect on the MDSC population. However, in this same model, anti-PD-L1 treatment increased CD8+ T cells and decreased both Tregs and MDSCs. A more complete mechanistic understanding of how the immune system responds to checkpoint inhibitors permits identification of appropriate models for monotherapy, as well as the rationale for designing drug combination studies. Data enhancing understanding of the above, along with tumor profiling on additional syngeneic models and more flow cytometry multiplexing, is expected from further ongoing research.


Inhibitors of inflammatory checkpoints, such as PD-L1 inhibitors, have demonstrated great promise in preclinical and clinical studies. This therapeutic paradigm focuses on controlling natural inflammatory checkpoints to stimulate an elevated inflammatory response against the tumor to increase anti-tumor inflammatory cell infiltrates in the tumor microenvironment or decrease inflammatory suppressor infiltrates. The proteins which control these processes can be found in the tumor cells, cells in the tumor microenvironment (TME), or in both locales. Positive cells are often assessed in a qualitative or semi-quantitative manner using immunohistochemistry and evaluation of a limited number of representative microscopy fields across a particular tissue compartment (tumor vs stroma) or the whole tissue area. However, the locale of the inflammatory suppressors such as PD-L1 may be more revealing than estimating the tumor-wide dispersion of an inflammatory cell type. Unfortunately, the intricate spatial relationships and the often complex distribution of inflammatory cells in tissues pose significant challenges for a meaningful evaluation.

We have developed an approach which can quantify these spatial relationship in a contextual, biologically meaningful score. Immunohistochemistry staining for PD-L1 in whole lung cancer tissue sections was performed, and our CellMap software was used to assess inflammatory cell distribution in the whole tissue sections. PD-L1 positive cells were quantified relative to: 1) the total number of cells in the tumor and stromal tissue compartments, and 2) the number of cells within a distance from the tumor/stroma interface. Interestingly, several unique PD-L1 distribution patterns relative to the tumor/stroma interface were observed in the sample cohort analyzed. Quantifying the distribution of PD-L1 positive cells as a function of distance from the tumor/stroma interface revealed distribution signatures, which could be used to differentiate between samples. In contrast, this differentiation of the same samples was not possible when PD-L1 cells were assessed relative to the total number of cells.
This study provided a novel method for assessing inflammatory cell type spatial distribution relative to a tissue feature, the tumor/stroma interface. The data suggested that unique spatial patterns of inflammatory cell type distribution could be used to uniquely stratify patients compared to existing quantitative methods. Taken together, this proof-of-concept study demonstrates a unique quantitative assessment of inflammatory cell infiltrates in tumors that could be used to gain new insights into inflammatory cell type distributions and interactions in tumors, inflammatory cell spatial responses to oncology therapies, and novel patient selection criteria for traditional and immuno-oncology therapeutics.


Current cancer biology acknowledges the key role of the immune system in tumor biology, and promise for the modulation of immune system in cancer treatment. The composition of the inflammatory cell populations in tissues is reflective of the overall state of the Tumor Micro-Environment (TME), and the identification of distinct inflammatory cell types may hold prognostic or predictive value. Immunohistochemistry allows for reliable identification of the cell constituents to facilitate analysis of the TME while remaining in the tissue context.

Establishing a quantitative paradigm for inflammatory cell types and subtype profiling requires unbiased and automated whole-tissue based quantitation methods, which are capable of spatial integration of multiple inflammatory cell markers across the whole tissue. While single slide fluorescent multiplex approaches can address this need, the use of difficult-to-implement wet assay strategies involving multiplexing 6-8 fluorescent markers on the same tissue section are difficult to implement in a global clinical diagnostic lab setting. To answer this need, we combined novel advents in Tissue Image Analysis (TIA) to integrate spatial expression of serial-section stained whole tissue clinical lung cancer specimens.

In this proof-of-principle study, we were able to superimpose specific locations of individual cell types onto 6 serial sections and evaluate different inflammatory cell types. We used serial sections of clinical lung specimens stained for six immune phenotypic markers (CD68, CD4, CD8, CD33, FoxP3, and CD11b) to illustrate a repertoire of inflammatory cell types. Our proprietary CellMap algorithm was utilized to identify, enumerate, and determine the precise location of individual inflammatory cells in tissues on cell-by-cell basis in the tumor microenvironment (TME). Our proprietary FACTS (Feature Analysis on Consecutive Tissue Sections) approach was used to integrate the spatial expression of individual markers onto a reference H&E slide, and/or adjacent slides. Using the aligned FACTS data and our proprietary MultivariateMap approach, we integrated the patterns of each marker based on immune cell type function and their location relative to each other and the tumor epithelial cells.

In this study, we demonstrated how spatial integration of immune cell markers in the context of whole tissues can be applied to the diagnostic setting. By creating a comprehensive landscape of the immune system state in the tissue biopsies, we were able to identify crucial patterns which represent function and role in immune system biology. These approaches provide a robust platform for immuno-oncology applications by providing information on the state of the immune system in cancer using approaches implementable in the clinic. The use of these approaches will benefit further understanding of cancer pathology, and can directly lead to the development of diagnostic tests with clinical utility.
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C110 Evaluation of immune cell populations in checkpoint inhibitor responsive syngeneic models. Sheri Barnes, Marcio Lasaro, Jacob Hauser, Robert J. Mullin, Aidan Synnott. Charles River Laboratories, Morrisville, NC.

Given the increasing success of cancer immunotherapies, we recognized a growing need for well-characterized preclinical models to enable drug discovery efforts. Syngeneic mouse tumor models offer advantages over conventional xenograft models for immunotherapy studies due to the presence of a fully functional immune system. We recently completed a comparative evaluation of the responsiveness of a collection of syngeneic models to two antibody based immunocheckpoint inhibitor therapeutics (anti-CTLA4, anti-PD1). This body of work resulted in the identification of several models responsive to checkpoint inhibition; CT26, Colon26, MC38, EMT-6, and A20. Our results clearly show differential tumor growth responses across this set of models following treatment with CTLA-4 and PD-1 targeted therapy. To further characterize these responses, we have performed a series of studies to identify and track immune cell populations by flow cytometry in blood, spleen, and tumor samples from mice harboring each responsive model. In these studies, we have investigated whether the modulation of CD4+, CD8+, regulatory CD4+ T cells (Treg), and myeloid derived suppressor cells (MDSC) populations correlates with the impact of immunocheckpoint inhibitors on tumor growth over time in untreated and challenged animals. These correlations of flow analyses with our antitumor data can point to the utility of cell-based endpoints as additional tools for evaluating combination outcomes.

C111 Safety profile and tumor response in patients with refractory superficial cancers treated with HF10, an oncolytic replication-competent HSV-1-derived intratumoral injectable, as monotherapy and combined with ipilimumab. Robert H.I. Andtbacka1, Kenneth F. Grossmann1, Hung T. Khong1, Maki Tanaka2, Richard S. Ungerleider3, Aislyn D. Boran3, Robert L. Ferris4. 1Huntsman Cancer Institute, Salt Lake City, UT; 2Takara Bio Inc., Kusatsu, Shiga, Japan; 3Theradex, Princeton, NJ; 4University of Pittsburgh Cancer Institute, Pittsburgh, PA.

Background: Here, we report on safety, recommended HF10 dose, viral shedding in body fluids and overall (all target lesions) and local (injected lesion) antitumor activity in patients (pts) with superficial tumors (13 squamous cell carcinoma, 9 malignant melanoma and 4 other tumor types) in a Phase 1 monotherapy study. In addition, we report on preliminary safety data and antitumor activity of HF10 + ipilimumab combination in an ongoing Phase 2 trial in pts with unresectable or metastatic melanoma.

Methods: In Stage 1 of the Phase 1 study, a single HF10 injection was administered to a superficial/cutaneous lesion; in Stage 2, up to 4 injections (≥ 2 weeks apart) were given. Adverse events (AEs) were assessed per CTCAE 3.0. Tumor responses were assessed per RECIST 1.0.

In the ongoing Phase 2 study, HF10 is injected into single or multiple tumors (at 1 x 107 TCID50/mL, up to 5mL depending on tumor size and number); 4 injections at 1-week intervals; then up to 15 injections at 3-week intervals. Four ipilimumab IV infusions (3 mg/kg; concurrent with HF10) are administered at 3-week intervals. AEs are assessed per CTCAE 4.0. Tumor responses are assessed per mWHO and iRC.

Results: Of 26 pts treated in the Phase 1 study, 24 pts had AEs. Nine (35%) pts had HF10-related AEs including chills (n=3), fatigue (n=2) and injection site reactions (n=6). There were no ≥ Grade 3 (G3) HF10-related AEs. HF10 was rapidly cleared from blood, saliva and urine. Pt HSV-1 serostatus did not affect AE profile or HF10 clearance. Of 24 evaluable pts at end of study (EOS; ≥ 10 wks after start of HF10), none had complete (CR) or partial response; 7 had local stable disease (SD); 8 had overall SD, and 6 of 9 melanoma pts had overall SD. Although there were no partial responders per RECIST, but 1 melanoma pt had a 45%
decrease in injected tumor size and another melanoma pt had 3 uninjected lesions disappear, despite overall SD. In long-term follow-up after EOS, 3 other melanoma pts had delayed responses in HF10-injected lesions: 1 pt with an injected thigh tumor had pathological CR on resection and remains disease free 1.5 yrs later, 1 pt with an injected submandibular lymph node (LN) had CR and remains disease free, and 1 pt had CR in an injected neck LN, but has slowly progressed in a lung metastasis. None of these 3 pts received any additional treatment after completing HF10 injections.

In the ongoing Phase 2, 24 pts have been treated and HF10-related AEs reported thus far are similar to those previously reported for HF10 monotherapy and none are ≥ G3. Of note, the commonly reported ≥ G3 immune-mediated ipilimumab-related events of colitis, hepatitis and endocrinopathies have not been observed. Four efficacy evaluable and 2 pts on-study < 12 wks (not yet evaluable) are showing a decrease in lesion size in response to treatment, with 2 documented local CRs.

Conclusions: Intratumoral HF10 serial injections are safe and well-tolerated, in monotherapy (Phase 1) and in combination with ipilimumab (preliminary Phase 2). HF10-related AEs include chills, fatigue and injection site reactions. Efficacy evaluation (overall and local) suggests that HF10 has both local and systemic antitumor activity in melanoma and can result in delayed responses. Assessment of HF10 as a potential new treatment for melanoma pts is ongoing in a Phase 2 study.

C112 Cabozantinib eradicates advanced murine prostate cancer by activating anti-tumor innate immunity. Akash Patnaik, Kenneth Swanson, Sabina Signoretti, Huihui Ye, Eva Csizmadia, Jesse Novak, Marina Gehring, Katja Helenius, Athalia Pyzer, Laleh Montaser, Lily Wang, Olivier Elemento, Elena Levantini, John Clohessy, John Asara, Kathleen Kelly, Pier Paolo Pandolfi, Jacalyn Rosenblatt, David Avigan, Steven Balk, Lewis Cantley. Beth Israel/Harvard Medical School, Boston, MA; Brigham & Woman's Hospital/Harvard Medical School, Boston, MA; Beth Israel Deaconess Medical Center, Boston, MA; Brigham & Woman's Hospital, Boston, MA; Massachussetts Institute of Technology, Cambridge, MA; Weill Cornell Medical College, New York, NY; National Cancer Institute, Bethesda, MD.

Several kinase inhibitors targeting aberrant signaling pathways in tumor cells have been deployed in cancer therapy. However, their impact on the tumor immune microenvironment remains poorly understood. The tyrosine kinase inhibitor cabozantinib showed striking responses in early phase clinical trials, particularly in cancer patients with bone metastases. Here we show that cabozantinib rapidly eradicates invasive, poorly differentiated PTEN/p53 deficient murine prostate cancer. This was associated with increased neutrophil chemotactic factor expression, including CXCL12 and HMGB1 production by tumor cells, and robust infiltration of neutrophils into the tumor. Critically, cabozantinib-induced tumor clearance in mice was abolished by antibody-mediated granulocyte depletion or HMGB1 neutralization or blockade of neutrophil chemotaxis with the CXCR4 inhibitor, plerixafor. Collectively, these results demonstrate that cabozantinib triggers neutrophil-mediated anti-tumor innate immune response that results in tumor clearance.

(Manuscript submitted to Science)
The monocarboxylate transporter 1 (MCT1) inhibitor AZD3965 triggers MCT4-dependent lactate accumulation and blocks pyruvate-lactate exchange in human cancer cells. Mounia Beloueche-Babari1, Slawomir Wantuch1, Markella Koniordou1, Harry G. Parkes1, Vaitha Arunan1, Thomas R. Eykyn1, Paul D. Smith2, Martin O. Leach1. 1Inst. of Cancer Research, London, United Kingdom; 2AstraZeneca, Macclesfield, United Kingdom.

Background: Monocarboxylate transporters (MCTs) are key modulators of lactate homeostasis and represent promising metabolic targets for molecular cancer therapeutics. The MCT1 inhibitor AZD3965 is now in clinical trial and understanding the impact of this drug on tumour cell metabolism may enable the discovery of pharmacodynamic (PD) biomarkers of target inhibition that will support the clinical development of such agents.

Since MCT1 mediates the bidirectional transport of lactate and other monocarboxylates including pyruvate, here we use NMR spectroscopy to investigate the effect of AZD3965 on a) intracellular lactate levels and b) hyperpolarized $^{13}$C-pyruvate-lactate exchange, as biomarkers for MCT1 inhibition in human cancer cells with varying MCT4 expression (predictive of resistance to AZD3965).

Materials and Methods: Human Raji (MCT4-) and Hut78 (MCT4 low (+)) lymphoma as well as HT29 (MCT4 high (+++)) colon carcinoma cells were treated with either 5nM or 500nM AZD3965 for 24h and levels of intracellular lactate determined by $^1$H NMR of cell extracts. Raji cells were also exposed to additional AZD3965 concentrations spanning InM-500nM. For $^{13}$C-pyruvate-lactate exchange studies, Raji cells were treated with either 5nM or 25nM AZD3965 for 24h. Cells were then incubated at 37°C in FBS-free medium and dynamic $^{13}$C NMR spectra acquired for 4 minutes with 2s intervals immediately after the addition of 10mM hyperpolarised $[1-^{13}C]$pyruvic acid and 10mM unlabelled lactate. The ratio of the area under the curve for the summed lactate and pyruvate signals ($Lac_{AUC}/Pyr_{AUC}$) was determined to estimate pyruvate-lactate exchange. Data represent mean±SE.

Results: 24h exposure to a low concentration of AZD3965 (5nM) led to increased intracellular lactate in MCT4- Raji and MCT4+ Hut78 human lymphoma cells to 2.65-fold and 10-fold respectively ($p=≤0.02$) while the effect in MCT4+++ HT29 human colon carcinoma cells was insignificant (167±31% of controls, $p=0.1$). Exposure to a high concentration of AZD3965 (500nM) increased intracellular lactate accumulation in HT29 cells albeit to a lesser degree than in Raji and Hut78 cells (4-fold (0.02) vs. 14 to 15-fold ($p=≤0.01$) in the lymphoma lines). This effect is consistent with the expected blockade of lactate release in cells with low or no MCT4 expression following MCT1 inhibition. Lactate build-up in Raji cells was AZD3965 concentration-dependent being observed with as little as 1nM (up 1.7-fold), reaching a maximum at 25nM (12-fold) and plateauing thereafter.

Analysis of the hyperpolarized $^{13}$C NMR data showed a significant decrease in $Lac_{AUC}/Pyr_{AUC}$ to 31±6% in 5nM and 19±2% in 25nM AZD3965-treated Raji cells relative to controls ($p<0.001$). These results are consistent with MCT1-mediated transport of $^{13}$C-pyruvate being a rate limiting step in the $^{13}$C NMR-observed pyruvate-lactate exchange.

Conclusions: Our data show that AZD3965 triggers intracellular lactate accumulation in a concentration- and MCT4 expression-dependent manner and inhibits $^{13}$C-pyruvate-lactate exchange (via blockade of $^{13}$C-pyruvate uptake). Intracellular lactate and hyperpolarized $^{13}$C-pyruvate-lactate exchange measurements are translatable to in vivo imaging studies and are therefore promising non-invasive metabolic biomarkers for AZD3965 and potentially other MCT1 inhibitors.

Lung cancer is the leading cause of cancer related death worldwide and adenocarcinoma is the most prevalent subtype. LKB1 (STK11) is the second most commonly altered tumour suppressor in adenocarcinoma and is inactivated in 17% of samples (1). LKB1 is a master kinase that phosphorylates up to 13 downstream targets involved in cell polarity and metabolism. It has been reported that LKB1 mutated cells are more sensitive to the metabolic inhibitor phenformin (2). We aimed to further investigate this by testing the toxicity of 3 metabolic inhibitors (metformin, phenformin and 2-DG) in LKB1 mutated (n=8) and LKB1 WT (n=4) NSCLC cell lines, as well as in isogenic cell lines with or without WT LKB1 expression (A549-wtLKB1, A549-mutLKB1).

There was a wide range of sensitivity [IC50 values: metformin 1.22 mM–34.37 mM; phenformin 0.01–1.39 mM; 2-DG 1.86–15.18 mM] to the inhibitors in the 12 NSCLC cell lines. The IC50 values for the LKB1 WT cell lines fell within the range of IC50 values for the LKB1 mutated cell lines. IC50 data was analysed using ANOVA with LKB1, KRAS, and TP53 mutation status as factors and interpreting the partial eta squared (η²) using Cohen’s rule of thumb to assess the effect size. By this analysis, LKB1 mutation status had a small effect in determining sensitivity to metformin or phenformin and TP53 mutation status had a large impact, with WT TP53 cells being more sensitive.

To further investigate the effect of TP53 status, we transfected isogeneic A549 cells (A549-wtLKB1, A549-mutLKB1) with a vector expressing SV40 large T antigen to inhibit TP53 function. Loss of TP53 function increased resistance in both A549-wtLKB1 (metformin IC50: 5.42 versus 1.92 mM, p < 0.001; phenformin IC50: 0.57 versus 0.02 mM, p <0.001) and A549-mutLKB1 (metformin IC50: 3.99 versus 1.10 mM, p < 0.001; phenformin IC50: 0.53 versus 0.01 mM, p = 0.04) cells. As TP53 is the most commonly mutated tumour suppressor gene in lung adenocarcinoma (46%) (1), our results suggest that TP53 mutation status may be an important factor when considering treatment of NSCLC with metabolic inhibitors.


Using isogenic colorectal cancer cell lines with either WT or mutant PIK3CA allele knockout, we demonstrated that CRCs with PIK3CA mutations are more sensitive to growth inhibition by CB-839. Remarkably, combination of CB-839 with 5-FU induces regression xenograft tumors from a CRC with a PIK3CA mutation, suggesting that this combinational therapy may be effective approach to treat CRC patients whose tumors harbor PIK3CA mutations.

Mechanistically, mutant p110α up-regulates gene expression of glutamate pyruvate transaminase 2 (GPT2) in CRC cells, thereby facilitate conversion of glutamate to α-KG. Using [13C5]-glutamine isotope-tracing technology, we showed that PIK3CA mutant CRCs produce more α-KG from glutamine to α-KG to replenish the tricarboxylic acid (TCA) cycle to generate ATP. Moreover, aminooxyacetate, which inhibits enzymatic activity of transaminases including GPT2, suppresses xenograft tumor growth of CRCs with PIK3CA mutations, but not CRCs with WT PIK3CA. Mutant p110α up-regulates the transcription of GPT2 through an AKT-independent PDK1-RSK2-ATF4 signaling axis. We showed that ATF4 is a transcription factor that activates GPT2 gene expression. We further demonstrated that mutant p110α activates RSK2 kinase through PDK1. Activated RSK2 then phosphorylates ATF4 at the serine residue 245, which in turn recruits deubiquitinase USP8 and protects ATF4 from ubiquitin-mediated degradation. Together, our data establish oncogenic PIK3CA mutations as a cause of glutamine addiction in CRCs and that targeting glutamine metabolism may be effective approach to treat CRCs with PIK3CA mutations.

Concomitant blockade of glucose uptake and paracrine HGF signaling uncovers the metabolic vulnerability of KRAS-mutant colorectal tumors. Alessia Mira, Paolo Michieli. Candiolo Cancer Institute, Candiolo, Turin, Italy.

Mutations in KRAS and over-expression of MET, the hepatocyte growth factor (HGF) receptor, are both negative prognostic factors for colorectal cancer (CRC). We studied the KRAS-MET interplay using human isogenic CRC cells with defined KRAS status and human HGF knock-in SCID mice, thereby reproducing species-specific HGF-MET paracrine signaling. We show that KRAS-mutant CRC cells display an extraordinary high glycolytic rate that makes them ‘addicted’ to glycolysis. When glucose availability is scarce or upon drug-mediated impairment of glucose metabolism, KRAS-mutant cells rapidly undergo apoptosis, unless ‘salvaged’ by micro-environment-borne HGF, which permits their survival by enhancing glucose influx, sustaining glycolysis and activating autophagy, thus improving energetic efficiency and preventing a mitotic catastrophe. In an orthotopic model of metastatic CRC using human HGF knock-in SCID mice and human KRAS-mutant CRC cells, combined pharmacological blockade of glucose metabolism and of HGF/MET signaling resulted in synthetic lethality and achieved superior tumor inhibition and metastasis suppression compared to metabolism-active drugs or HGF/MET inhibitors alone. In the same system, paracrine HGF signaling also protected KRAS-mutant tumors against anti-angiogenic therapy, which caused more dramatic and less specific metabolic stress by depriving tumors of both nutrients and oxygen. However, in contrast to selective glucose metabolism impairment, anti-angiogenic therapy exacerbated tumor hypoxia, promoted HGF-dependent metastatic dissemination and gave rise to acquired resistance. These results indicate that selective impairment of glucose metabolism is a much safer and effective approach to target KRAS-mutant CRC metabolic vulnerability compared to angiogenesis inhibition. They also show that microenvironment-borne HGF is a major source of resistance to anti-tumor therapies that cause metabolic stress to cancer cells. Considering that angiogenesis inhibition is the only biological therapy currently available for KRAS-mutant tumors, the data generated by this study open new perspectives for the treatment of this highly aggressive cancer type.
Despite global advances in cancer detection and treatment in some indications, the early diagnosis and overall survival rate for pancreatic cancer (PanCa) remains dismal. Thus, there is a critical need for novel therapeutics that may combine well with standard-of-care therapy or work through novel mechanisms. Given that most pancreatic tumors exhibit a highly metabolic phenotype, we examined the effects of BPM 31510 employing in vitro and in vivo PanCa models. BPM 31510 is a metabolic-modulating agent that reverses the Warburg effect and is currently in clinical development for solid tumors alone and in combination with chemotherapy. Determination of BPM 31510 IC50 values in vitro demonstrated the PanCa cell lines MIA PaCa-2 and Panc-1 cells were significantly more sensitive to BPM 31510 (IC50 = 137 and 455 μM, respectively) compared to primary fibroblasts (IC50 = 1537 μM). IC50 and IC90 doses of BPM 31510 also decreased the viable cell population while concomitantly increasing Annexin V- and PI-positive populations in both PanCa cell types, indicating BPM 31510 induces programmed cell death. Furthermore, in combination with gemcitabine (0.1-5 μM), BPM 31510 (100 μM) decreased cell viability by more than 75% compared to either treatment alone. In vivo, treatment of MIA PaCa-2 tumor-bearing mice with increasing doses of BPM 31510 (0.5-50 mg/kg IP, 3X/week) significantly improved median survival in a dose-dependent manner, with the highest dose extending median survival by more than 36 days compared to saline control. Moreover, while median survival of MIA PaCa-2 tumor-bearing mice treated with BPM 31510 (50 mg/kg IP, 1X/day) or gemcitabine (150 mg/kg IV, 1X/week, given on cycles, 3 weeks on 1 week) monotherapy was 77 and 63 days, respectively, combination treatment resulted in median survival improvement to 113.5 days. Examination of alternative dosing regimens revealed that more frequent dosing of BPM 31510 (2X or 3X/day) alone and in combination with gemcitabine further extended median survival in this model. The preliminary mechanistic insight into additive efficacy of combination treatment was explored in vitro. BPM 31510 treatment alone significantly altered multiple aspects of mitochondrial function in MIA PaCa-2 cells, indicating that BPM 31510-driven bioenergetic alterations are separate from the effects of gemcitabine. Hence, these data demonstrate that BPM 31510 has a potent anti-cancer activity alone and in combination with standard-of-care chemotherapy in preclinical PanCa models.
0.27±0.06 μM). Across a panel of 131 human kinases, the inhibitor showed minimal off-target effects (only 5 kinases were inhibited more than 20% at a concentration of 10 μM). ICL-CCIC-0019 potently inhibited growth of a panel of 60 cancer cell lines with median GI50 of 1.12 μM. Importantly, proliferation of normal cells was only minimally affected (MCF-10A and ST-Tib: GI50 30-120 μM). ICL-CCIC-0019 decreased phosphocholine levels and the fraction of labeled choline in lipids, and induced G1 arrest, endoplasmic reticulum stress and apoptosis. Changes in phosphocholine cellular levels following treatment could be detected non-invasively in tumor xenografts by 18F-fluoromethyl-[1,2-2H4]-choline positron emission tomography. Pharmacokinetic modeling revealed that the macro parameter Ki denoting the net irreversible uptake rate was significantly decreased in tumor after 48 hours (Ki (1/min): control, 0.0054±0.00060; ICL-CCIC-0019, 0.0032±0.00064), confirming in vivo target inhibition. This resulted in potent antitumor activity in HCT116 xenografts. We further reveal a previously unappreciated effect of choline metabolism on mitochondria function. Comparative metabolomics demonstrated that phosphatidylcholine pathway inhibition leads to a metabolically stressed phenotype analogous to mitochondria toxin treatment but without reactive oxygen species activation. Drug treatment decreased TCA cycle activity, oxygen consumption rate and elevated extracellular acidification rate. This was associated with a reduction of citrate synthase expression and AMP kinase activation. Glucose and acetate uptake were increased in an attempt to overcome the metabolic stress. This study indicates that choline pathway pharmacological inhibition is a valid therapeutic strategy and critically affects the metabolic function of the cell beyond reduced synthesis of phospholipids.

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Monoclonal Antibodies

C119 Investigation of the anti-angiogenic effects of abituzumab in patients with colorectal or ovarian cancer and liver metastases using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI). Josep Tabernero1, Geoffrey Parker2, Andrew Clamp3, Elena Elez1, Nerissa Mescallado3, Rodrigo Dienstmann1, Tamara Sauri1, Jurjees Hasan3, Jose J. Mateos1, Rolf Bruns4, Claude Gimmi4, Gordon C. Jayson5. 1Vall d’Hebron University Hospital, Vall d’Hebron Institute of Oncology (VHIO), Barcelona, Spain; 2Centre for Imaging Sciences, University of Manchester, Manchester, United Kingdom; 3The Christie NHS Foundation Trust and Institute of Cancer Sciences, University of Manchester, Manchester, United Kingdom; 4Merck KGaA, Darmstadt, Germany.

Background: Integrins promote cell survival, metastasis, and angiogenesis, with integrins αvβ3 and αvβ5 promoting neovascularization. Abituzumab (EMD 525797) is a humanized monoclonal IgG2 antibody that specifically binds to the αv integrin subunit to inhibit αv integrin-mediated functions. It has shown antitumor activity in preclinical models and clinical trials. We investigated the tolerability and potential anti-angiogenic activity of abituzumab using DCE-MRI.

Methods: This phase I study recruited patients (pts) ≥18 years with liver metastases (3-10 cm in diameter) of either colorectal (CRC) or ovarian cancer (OC) who had failed standard therapy. Pts received abituzumab 250, 500, 1,000, or 1,500 mg IV q2w, with ≥6 pts per dose. The decision to escalate the dose was based on the occurrence of dose-limiting toxicity (DLT). DCE-MRI scans were performed 24 and 96 hours after the first abituzumab dose, 1 week after first dose, and immediately prior to the second dose to identify changes in parameters including Ktrans, extracellular/extravascular volume, and blood plasma volume during one cycle of therapy. DCE-MRI scan data were analyzed centrally. Tumor response was evaluated every 6 weeks.
Primary objectives were to assess the tolerability of abituzumab and to investigate vascular and volumetric responses to abituzumab using DCE-MRI. Secondary objectives included characterization of the pharmacokinetics of abituzumab and its effect on exploratory pharmacodynamic markers, including tumor markers.

Results: 41 pts were enrolled (CRC, n=30; OC, n=11). All pts had received ≥1 prior anticancer treatment. Four of 31 pts included in the dose-escalation analysis set had DLTs: myocardial ischemia (250 mg, n=1/6), intracranial hemorrhage (500 mg, n=1/12), and drug hypersensitivity (1,500 mg, n=2/7). With the exception of drug hypersensitivity, the nature and incidence of adverse events (AEs) were similar between the dose levels. The most frequently reported treatment-related AEs were fatigue (12.2%) and headache (12.2%). Of 32 patients with available response information, 3 with OC had a best overall response of stable disease (SD) lasting ≥6 weeks (2 treated with abituzumab 500 mg, 1 with abituzumab 1,000 mg); one 73-year-old pt with high-grade serous OC who received abituzumab as 5th-line therapy had SD for 33 weeks and a CA-125 response. No complete or partial responses were observed. No trends in differences in mean DCE-MRI parameters between abituzumab doses and no vascular or volumetric responses to abituzumab were observed, although alterations in DCE-MRI parameters were noted in individual patients. Small changes in IAUC60, Ktrans, and blood plasma volume were observed between Week 1 Day 5 and Week 2 Day 1 that may have been due to abituzumab-induced effects, but did not correlate with clinical activity (SD).

Conclusions: This phase I trial has confirmed the tolerability of abituzumab, although hypersensitivity reactions represent a new event that warrants further investigation. The occurrence of SD in pts with OC with large liver metastases suggests that abituzumab has activity. However, there was little evidence of a clear effect of abituzumab on tumor vasculature, suggesting limited dependence on neovascularization in advanced tumors.

C120  Phase I study of the safety and tolerability of LJM716 in Japanese patients with advanced solid tumors. Taito Esaki1, Hisanobu Oda1, Tatsuhiro Kajitani1, Takayuki Kobayashi2, Junichi Tomomatsu2, Tomoyuki Kakizume2, Takahiro Watanabe3, Hiromi Takeuchi3, Koichi Fukino3, Shunji Takahashi3.1Department of Gastrointestinal and Medical Oncology, National Kyushu Cancer Center, Fukuoka, Japan; 2Department of Medical Oncology, Cancer Institute Hospital of Japanese Foundation for Cancer Research, Tokyo, Japan; 3Novartis Pharma KK, Tokyo, Japan.

Background: Human epidermal growth factor receptor 3 (HER3) is implicated in tumor growth, proliferation, drug resistance, and metastasis. LJM716 is a fully humanized anti-HER3 IgG1 monoclonal antibody with single-agent and combination anti-tumor activity in HER2-amplified and neuregulin-expressing xenografts. This open-label dose escalation Phase I study evaluated the safety and tolerability of single-agent LJM716 in Japanese patients (pts) with HER2+ advanced/metastatic breast (BC) or gastric cancer (GC), and recurrent/metastatic esophageal squamous cell carcinoma (ESCC) or squamous cell carcinoma of the head and neck (SCCHN) regardless of HER2 status.

Methods: Pts (aged ≥18 years, ECOG PS 0–2) received intravenous (IV) once-weekly (QW) LJM716 in 28 day cycles. The primary objective was to determine the maximum tolerated dose (MTD) and/or recommended dose (RD). Secondary objectives included safety and tolerability, preliminary anti-tumor activity, and pharmacokinetics. Dose escalation decisions were made based on a synthesis of all relevant data, guided by an adaptive Bayesian logistic regression model (BLRM) on dose limiting toxicities (DLTs).

Results: At the data cutoff date of Jun 3, 2015, 12 pts (SCCHN [n=2], ESCC [n=2], and HER2+ BC [n=6] or GC [n=2]) were enrolled (median age 58 years, 50% male, 58% ECOG PS 0). Pts were treated in 3 dose cohorts...
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of 10–40 mg/kg QW; the median duration of exposure to LJM716 was 14.0 weeks (range 4.0–48.1). No DLT was reported during Cycle 1; at 40 mg/kg QW 1 pt experienced a DLT of Grade (Gr) 3 pneumonia aspiration during Cycle 2. The BLRM with overdose control supported the tolerability of LJM716 up to 40 mg/kg QW based on DLTs occurring during Cycle 1. However, DLTs occurring after Cycle 1 were also clinically considered, and 40 mg/kg QW was declared as the RD in Japanese pts; the MTD was not reached. One or more adverse event (AE) suspected as drug related were experienced by 10 (83%) pts; most commonly (>25%) diarrhea (6 pts [50%]), stomatitis, paronychia, fatigue, and pyrexia (3 pts [25%] each). Four pts (33%) experienced ≥1 Gr 3/4 drug-related AEs (all at 40 mg/kg QW): pneumonia aspiration and neutropenia (1 pt [8%] each) and lymphocyte count decreased (2 pts [17%]). Serious AEs were experienced by 2 pts (17%); Gr 2 nausea and Gr 1 vomiting (not suspected as drug related) and Gr 3 pneumonia aspiration (suspected as drug related). One pt died within the follow up period after the last dose of study drug due to disease progression. LJM716 plasma concentration increased with dose, and mean AUClast and Cmax were similar to those found in Western pts. There were no complete or partial responses; stable disease was reported in 6 (50%) pts.

Conclusion: LJM716 was well tolerated with a manageable safety profile, and the RD of LJM716 was established at 40 mg/kg QW IV in Japanese pts - the same RD as determined in Western pts in a separate clinical trial. Clinical trials identifier: NCT01911936.

CI21A non-randomized, open-label, single-arm, phase 2 study of LY2875358 in Asian patients with MET diagnostic positive, advanced gastric cancer. Hyun Cheol Chung1, Taroh Satoh2, Do-Youn Oh3, Se Hoon Park4, Shigenori Kadowaki5, Volker Wacheck6, Ayuko Yamamura7, Kazunori Uenaka7, Xuejing Aimee Wang6, Sameera R. Wijayawardana6, Toshihiko Doi8,1Yonsei Cancer Center, Seoul, Korea; 2Osaka University Hospital, Suita, Japan; 3Seoul National University Hospital, Seoul, Korea; 4Samsung Medical Center, Seoul, Korea; 5Aichi Cancer Center Hospital, Nagoya, Japan; 6Eli Lilly and Company, Indianapolis, IN; 7Eli Lilly Japan K.K, Kobe, Japan; 8National Cancer Center Hospital East, Kashiwa, Japan.

Introduction: MET is expressed in gastric cancer and associated with poor clinical outcome. LY2875358 (LY) is a humanized immunoglobulin G4 (IgG4) monoclonal bivalent antibody blocking ligand-dependent and independent MET signaling. In preclinical studies, LY showed single agent anti-tumor activity for MET amplified gastric cancer in xenograft models. Based on these results, a non-randomized, multicenter, single-arm, open-label, Phase 2 study was conducted to evaluate the antitumor activity of LY in patients (pts) with MET diagnostic positive (+), advanced gastric or gastroesophageal junction (GEJ) adenocarcinoma.

Methods: Pts with MET diagnostic (+), advanced gastric or GEJ adenocarcinoma, who had received 2 prior chemotherapies, were administered LY 2000 mg as flat dose intravenously every 2 weeks (Q2W) on a 28 day cycle. MET diagnostic (+) tumor status was determined by immunohistochemistry (IHC). The primary objective was to evaluate the activity of LY in terms of progression-free survival (PFS) rate at 8 weeks (+ 3 days). Secondary objectives were to assess other efficacy variables (eg overall response rate, disease control rate [DCR], PFS, overall survival [OS]), toxicity and safety profile of LY, and pharmacokinetics (PK). The exploratory objectives included evaluation of pharmacodynamics, pharmacogenomics, and exploratory biomarkers.

Results: Tumor samples of 65 pts were screened for MET expression by IHC and 15 pts (23.1%) with MET diagnostic (+) were enrolled in this study. Fifteen pts (5 female, 10 male) from Asia (Japan 8, Korea 7) with a median age of 63 years (range 39-74) were enrolled. PFS rate at 8 weeks was 47% (70% Confidence Interval [CI]: 33%, 59%). There was no partial response according to RECIST, while shrinkage of tumor size was
observed in 3 out of 15 pts. DCR was 40.0%, with stable disease shown in 6 out of 15 pts. Median PFS was 8.3 weeks (95% CI: 4.1, 12.1) with stable disease for up to a maximum of 37.1 weeks. Median OS was 17.1 weeks (95% CI: 6.3, Not Available). A total of 12 pts (80%) experienced at least 1 LY-related treatment-emergent adverse event (TEAE). Common LY-related TEAE (all grades) included constipation and hypoalbuminemia (3 pts [20%] each). LY-related TEAEs with Grade ≥3 were hyponatremia and hyperuricemia (2 events in 1 patient), and hyperkalemia (1 patient). Serious adverse events were reported in 6 patients, none of which was related to LY. There was no TEAE leading to death or study treatment discontinuation. PK profiles were similar to those observed in previous studies of LY monotherapy, which were conducted in the United States, and the majority of patients were Caucasian. Exploratory biomarker analysis (IHC data) will be presented at the venue.

Conclusion: LY 2000 mg Q2W showed a well-tolerated safety profile with a limited single agent activity in heavily pretreated patients with MET diagnostic (+), advanced gastric or GEJ adenocarcinoma.

C122 A randomized, multicenter, open-label, phase 2 study of paclitaxel-carboplatin (PC) chemotherapy plus necitumumab (IMC-11F8/LY3012211) versus PC alone in the first-line treatment of patients (pts) with stage IV squamous non-small cell lung cancer (sq-NSCLC). David Spigel1, Alexander Luft2, Rodryg Ramlau3, Mazen Khalil4, Joo-Hang Kim5, Carlos Mayo6, Henrik Depenbrock7, Grace Chao6, Coleman Obasaju8, Ronald Natale8. 1Sarah Cannon Research:Tennessee Oncology PLLC, Nashville, TN; 2Leningrad Regional Clinical Hospital, St. Petersburg, Russian Federation; 3Poznan University of Medical Sciences, Poznan, Poland; 4Clayton Clinic, Jonesboro, AZ; 5Yonsei Cancer Center, Seoul, Korea; 6Eli Lilly and Company, Bridgewater, NJ; 7Lilly Deutschland GmbH, Bad Homburg, Germany; 8Eli Lilly and Company, Indianapolis, IN; 9Cedars Sinai Medical Center, Los Angeles, CA.

Background: Necitumumab (N) is a 2nd-generation human IgG1 anti-EGFR monoclonal antibody. In a phase 3 trial (SQUIRE) conducted in the first-line setting, N combined with gemcitabine plus cisplatin improved overall survival (OS) in pts with sq-NSCLC compared with chemotherapy alone. PC, another standard of care was selected for this exploratory phase 2 study.

Materials and Methods: Pts with stage IV sq-NSCLC, stratified by ECOG PS (0 vs. 1) and sex (females vs. males) were randomized 2:1 and received either PC (P=200 mg/m² iv, day 1; C=AUC 6 iv, day 1) plus N (800 mg iv, days 1 and 8) (PC+N arm), or PC alone (PC arm) every 21 days for up to 6 cycles. PC+N pts with a response of stable disease or better continued on N alone until progressive disease or intolerable toxicity. Rash prophylaxis was permitted from the beginning of the trial. The primary endpoint was objective response rate (ORR) based on RECIST 1.1. Secondary endpoints included progression-free survival (PFS), OS, and safety. Sample size and randomization allowed for a 95% confidence interval estimate of ORR for Arm PC+N with a width ≤20 percentage points. Final analysis was performed after a minimum of 98 OS events had been observed. No formal hypothesis testing was planned; the study was not powered to show a statistically significant effect for PFS or OS.

Results: 167 pts were randomized (n=110, PC+N; n=57, PC). Baseline characteristics were balanced between PC+N and PC, respectively, including males (79% and 77%), ECOG PS 0 (16% and 19%), and PS 1 (85% and 81%). Exposure to chemotherapy was similar in both arms; median dose intensity (DI) for P was 98% vs 95% (PC+N vs PC), and DI for C was 100% vs 95%, respectively, and DI for N was 96%. 53% of PC+N pts continued N alone for a median of 4 additional cycles (range 2.0-7.5). The addition of N to PC resulted in an ORR of 48.9% vs 40.0%. Disease control rate was 87.2% vs 84.0% in PC. PFS HR was 1.0 (mPFS 5.4 vs 5.6 months). Median OS was 13.2 vs 11.2 months (HR=0.83, p=0.379). There was a higher rate of events for the
PC+N arm during the first 4 months, with a later trend toward improved survival after 4 months. Most frequently reported SAEs were pneumonia (12.3% vs. 5.5%) and febrile neutropenia (5.7% vs. 3.6%). Grade ≥3 AEs typically associated with EGFR mAbs that showed a >2% increase were hypomagnesemia (5.7% vs 0) and rash (2.8% vs 0). Any grade venous thromboembolic events occurred in 3.8% of patients in the PC+N group vs 3.6% of patients in the PC group. Infusion related reactions of any grade occurred in 3.8% of patients in the PC+N group vs 9.1% in the PC group.

Conclusions: The addition of N to PC resulted in an increased ORR compared to PC alone, indicating an add-on effect. The lower incidence of grade 3 rash observed here (2.8%) versus SQUIRE (7%) may be due to the permission of first cycle rash prophylaxis (SQUIRE no rash prophylaxis permitted prior to cycle 2). The safety profile in this study is consistent with the expected safety profile of an EGFR mAb administered in this combination in the sq-NSCLC population. Clinical trial information: NCT01769391.


Introduction: Folate receptor alpha (FOLR1) is a member of the folate transporter family expressed on normal tissues and overexpressed in multiple types of tumors, such as ovarian cancer, uterine cancer, non-small cell lung cancer, gastric cancer, breast cancer and kidney cancer. Currently, several clinical trials of FOLR1-targeting drugs [conventional IgG1 antibodies, which exhibit antibody-dependent cellular cytotoxicity/complement-dependent cytotoxicity (ADCC/CDC) activities, folic acid or antibody-drug conjugates and vaccines] have been conducted for ovarian and lung cancer. Therefore, FOLR1 is a remarkable target for cancer therapy under ongoing investigation. AccretaMab® technology involves combining both the POTELLIGENT®, a clinically validated ADCC-enhanced technology, and COMPLEGENT®, a new CDC-enhanced technology, systems to result in a superior technology for enhancing the killing activity of antibodies. KHK2805 is a novel humanized and CDR-altered anti-FOLR1 antibody developed with AccretaMab® technology. In this study, we evaluated the anti-cancer activity of KHK2805 in preclinical ovarian cancer models, both in vitro and in vivo, and confirmed the safety profile of KHK2805 in cynomolgus monkeys, since KHK2805 cross-reacts to cynomolgus monkey FOLR1.

Materials and Methods: The binding kinetics of KHK2805 against recombinant FOLR1 (rFOLR1) were measured using the Biacore system. The epitope was determined with an ELISA against rFOLR1s. The in vitro ADCC and CDC activities against FOLR1-positive ovarian cancer cells were evaluated using PBMCs and serum from healthy volunteers. The in vivo anti-tumor activity of KHK2805 was examined using a SCID mouse model. The safety profile of KHK2805 was evaluated in cynomolgus monkeys.

Results: KHK2805 induced potent ADCC and CDC activities against FOLR1-positive ovarian cancer cells. The ADCC activity of KHK2805 was significantly higher than that of the conventional anti-FOLR1 antibody. Furthermore, KHK2805 showed a potent ADCC activity against ovarian cancer cells with a low FOLR1 expression or low folic acid-uptake activity, which may be difficult to target with current FOLR1-targeting drugs. The results also showed that the markedly higher ADCC activity of KHK2805 was caused by its super-high affinity, unique epitope and use of AccretaMab® technology. In addition, the CDC activity of KHK2805 was also clearly higher than that of the conventional anti-FOLR1 antibody. This indicates that the higher CDC activity of KHK2805 is due to the application of protein engineering of CDR alterations and
AccretaMab® technology. Moreover, the potent anti-tumor activity of KHK2805 was observed in a peritoneal dissemination model in SCID mice. Finally, we completed preliminary safety experiments with KHK2805. A repeated-dose toxicity study of KHK2805 (weekly 100 mg/kg for 4 weeks, intravenously) showed an acceptable tolerability profile in cynomolgus monkeys.

Conclusions: KHK2805 may be a promising novel anti-FOLR1 therapeutic agent with a potent anti-tumor activity and tolerable safety profile for patients with the FOLR1 expression.


Introduction: Folate receptor alpha (FOLR1) is a folate transporter expressed in many cancers, including ovarian cancer. Currently, several clinical trials of FOLR1-targeting drugs [conventional IgG1 antibodies, which exhibit antibody-dependent cellular cytotoxicity/complement dependent cytotoxicity (ADCC/CDC) activities, folic acid or antibody-drug conjugates and vaccines] have been conducted for ovarian and lung cancer. Therefore, FOLR1 is a remarkable target for cancer therapy under ongoing investigation. We established KHK2805, a novel anti-FOLR1 monoclonal antibody, using AccretaMab® technology to enhance both ADCC and CDC activities. Translational research (TR) using clinical samples is essential for determining whether a novel drug shows potent efficacy in clinical studies. In this study, we evaluated the anti-cancer activity of KHK2805 using malignant ascites and serum samples from patients with ovarian cancer. In addition, the FOLR1 expression was evaluated immunohistochemically using ovarian cancer tissues.

Materials and Methods: An autologous ADCC assay was conducted using cells from the malignant ascites of ovarian cancer patients, in which both malignant cells (target cells) and immune cells (effector cells) were present. Similarly, the CDC activity was evaluated using supernatant of the malignant ascites obtained from the patients. Furthermore, a CDC assay using the serum of ovarian cancer patients was conducted. An immunohistochemical protocol was established using KM4193, the parental rat antibody of KHK2805, and formalin-fixed, paraffin-embedded ovarian cancer samples were immunohistochemically stained with KM4193.

Results: KHK2805 showed potent ADCC activity against FOLR1-positive ovarian cancer cells in the autologous setting using the malignant ascites samples of the ovarian cancer patients, showing a clearly higher activity than that of the conventional anti-FOLR1 antibody. In addition, the CDC activity of KHK2805 was higher than that of the conventional anti-FOLR1 antibody under conditions using the supernatant of malignant ascites or serum from the ovarian cancer patients. Therefore, KHK2805 is thought to have markedly higher killing activity against tumor cells in patients with ovarian cancer. An immunohistochemical examination of the FOLR1 expression showed that the ovarian cancer tissues were positively stained with KM4193.

Conclusions: TR using clinical samples from patients with ovarian cancer demonstrated that KHK2805 may be a promising novel anti-FOLR1 ovarian therapeutic agent with a potent antitumor activity.
C125  Preclinical pharmacodynamic biomarker and combination strategy of RG7386, a novel FAP-DR5 bispecific antibody for targeting solid tumors. Thomas Friess¹, Ann-Marie Broeske¹, Stefanie Lechner¹, Esther Abraham¹, Gabriele HoelzlWimmer¹, Hadassa Sade¹, Peter Bruenker², Oliver Krieter¹. ¹Roche Diagnostics GmbH, pRED Innovation Center Penzberg, Penzberg, Germany; ²Roche Glycart AG, pRED Innovation Center Zürich, Zürich, Switzerland.

Introduction: RG7386 is a novel bispecific antibody, binding with high affinity to fibroblast activation protein (FAP) and with low affinity to death receptor 5 (DR5). Avidity-driven binding of the bispecific antibody mediates hyper-clustering of DR5 thus triggering tumor cell death. Induction of FAP dependent apoptosis translated into strong efficacy in vivo using patient derived xenografts thereby proposing RG7386 as an attractive therapeutic approach for the treatment of stroma rich FAP-positive solid tumors as well as FAP positive sarcomas. However, thus far DR5 targeting strategies failed to show clinical efficacy likely due to lack of hyperclustering and intrinsic resistance mechanisms. Preclinical translational studies were conducted to demonstrate the on-target mode of action, to ensure maximal activity and to guide pharmacodynamic (PD) analysis to unravel potential resistance mechanisms.

Material and Methods: Based on strong anti-tumor efficacy of RG7386 alone and in combination with irinotecan in a colorectal cancer (CRC) cell line based xenograft model (DLD-1) co-injected with fibroblasts a kinetic study was designed. Tumors were explanted 6, 16, 72 and 168 hours after RG7386 single agent treatment and harvested for immunohistochemical (IHC) and ELISA based protein analysis of apoptosis markers, such as cleaved caspase 3 (cc3), cleaved PARP and activated caspase 8 and 9. Additionally, PD effects of RG7386 treatment as single agent and in combination with doxorubicin were investigated in a FAP positive desmoplastic melanoma cell line derived model (LOX-IMVI).

Results: We observed significant time-dependent induction of apoptosis upon treatment with RG7386 by IHC and ELISA in xenograft tumors expressing FAP in stroma or on tumor cells directly. High, transient levels of apoptosis markers such as cc3 were observed by IHC early after treatment compared to vehicle control. Analysis of equivalent tissue lysates by ELISA revealed also rapid induction of cc3, cleaved PARP and activated caspase 8 and 9 in monotherapy in the DLD-1 CRC xenograft model which was superior when given together with doxorubicin in the LOX-IMVI desmoplastic melanoma model.

Conclusion: We identified that RG7386 strongly induces tumor cell apoptosis in vivo shortly after injection independently if FAP was expressed on tumor stroma or at tumor cells and discovered optimal pharmacodynamic markers and time points for sampling and analysis. As a result, early clinical trials of RG7386 will be designed to increase the therapeutic potential by choosing the right combination partner and to ensure demonstration of the postulated mode of action by pharmacodynamic data.


Breast cancer is a heterogeneous disease comprised of various subtypes based on pathology and molecular profiling. Expression of hormone receptors (HR) and HER2 biomarkers are important determinants of therapy choice, due to the established role of these proteins as drivers of disease. Prolactin Receptor (PRLR) is a type 1 cytokine receptor that is expressed on a subset of breast cancers and may contribute to pathogenesis. Functionally, activation of PRLR by the hormone ligand Prolactin (PRL) induces PRLR dimerization and signaling resulting in cell proliferation and differentiation. While PRLR is expressed at low
levels in some normal human tissues including the mammary gland, it is relatively overexpressed in ~25% of human breast tumors and importantly, is rapidly internalized upon binding of anti-PRLR antibodies.

We developed an anti-PRLR antibody-drug conjugate (ADC), PRLR ADC, to target PRLR positive breast cancer. PRLR ADC is comprised of a fully human high affinity function-blocking anti-PRLR IgG1 antibody conjugated via a non-cleavable SMCC linker to the cytotoxic maytansine derivative DM1. Both unconjugated anti-PRLR antibody and the PRLR ADC block PRL mediated activation in vitro and induce rapid internalization of the receptor into lysosomes. PRLR ADC induces potent cell cycle arrest and cytotoxicity in several PRLR-expressing cell lines.

The in vivo efficacy of PRLR ADC was explored in breast cancer cell line xenograft models expressing both endogenous PRLR (MCF7, T47D) or transfected receptor (MCF7/PRLR). Treatment of tumor bearing SCID (T47D) or NCr Nude (MCF7) animals was initiated approximately 15 days post implantation of cells where tumor volumes averaged 150-200 mm³. In both T47D and MCF7/PRLR xenograft models, where PRLR is expressed highly, single or multiple (once weekly x 3) doses of 2.5-15 mg/kg resulted in significant inhibition of tumor xenograft growth. In the MCF7 model that expresses low levels of PRLR, inhibition and regression of tumors was observed at 10 and 15 mg/kg dose levels. In all models, higher doses resulted in greater and more prolonged repression of tumor growth. Conjugation of DM1 to anti-PRLR antibody was required for efficacy, as unconjugated antibody had no effect on tumor growth. Anti-tumor efficacy of PRLR ADC was also assessed in NSG mice bearing breast cancer Patient Derived Xenograft (PDXs) tumors with moderate and heterogeneous expression of PRLR. Treatment was initiated 21 days after implantation of the PDX tumors where the average tumor volume was ~500mm³. Anti-tumor efficacy was observed following 10 or 20 mg/kg PRLR ADC dosed once weekly x 4.

These studies demonstrate the promising anti-tumor activity of the PRLR ADC against PRLR positive breast cancers and support the continued development of this agent.

C127 Chimeric antibody chLpMab-7 targeting human podoplanin suppresses pulmonary metastasis via ADCC and CDC activities. Yukinari Kato, Mika K. Kaneko. Tohoku University, Sendai, Japan.

Background: Podoplanin (PDPN), a platelet aggregation-inducing transmembrane glycoprotein, is expressed in a variety of tumors, and binds to C-type lectin-like receptor-2 (CLEC-2). Overexpression of PDPN is involved in invasion and metastasis. Several anti-PDPN monoclonal antibodies (mAbs) such as NZ-1 showed antimitastatic activities by binding to platelet aggregation-stimulating (PLAG) domain of PDPN. Recently, we developed a novel mouse anti-PDPN mAb, LpMab-7 using the cancer-specific mAb (CasMab) method. LpMab-7 is more sensitive than anti-PLAG domain mAbs. Methods: We generated and characterized a mouse-human chimeric anti-PDPN mAb, chLpMab-7. We investigated antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) activities in vitro. Furthermore, we evaluated the in vivo efficacy of chLpMab-7 using both xenograft models and experimental metastasis models. In xenograft models, PDPN-expressing CHO (CHO/hPDPN) or a lung cancer cell line PC-10 was engrafted into nude mice. In experimental metastasis models, CHO/hPDPN cells were inoculated into tail vein of the nude mice. Results: ChLpMab-7 antibody showed ADCC and CDC activities against CHO/hPDPN, glioblastoma, mesothelioma, or lung cancer cell lines. Treatment with chLpMab-7 abolished both tumor growth in xenograft and spontaneous pulmonary metastasis. ChLpMab-7 exhibited dramatic suppression of experimental pulmonary metastasis, even when it was injected five days after the inoculation of tumor cells. Conclusion: ChLpMab-7 suppressed tumor development and hematogenous metastasis via ADCC/CDC activities. PDPN should be useful as a novel antibody-based therapy.

Background: The Notch pathway is an evolutionarily conserved cell-signaling system that plays an important role in both physiologic and pathologic conditions such as embryonic development and cancer. In the latter, Notch signaling has been reported to promote tumor growth by regulating different aspects of tumor biology such as cell survival, proliferation, angiogenesis, and stemness. Because of its importance, targeting of this pathway has been attempted via both small molecules (γ-secretase inhibitors; GSIs) and neutralizing antibodies (against individual Notch receptors or DLL4 ligand) but limitations still hamper the clinical use of such therapeutics, generally due to pathway complexity and toxicities caused by complete pathway inhibition (GSIs). Targeting Notch ligands such as Jagged1 (JAG1), offers the opportunity to selectively block specific elements of the pathway important in tumor biology thus avoiding normal tissue toxicities.

Methods: Structural studies defined a region of JAG1 (DSL + EGF1-3) that bound Notch1, which was used as an immunogen. Using classical hybridoma technology we have generated and subsequently characterised a panel of monoclonal antibodies (mAbs) against the JAG1 ligand, both in vitro (cell signaling and cell biology assays) and in vivo (tumor xenografts in both mice and rats).

Results: Four functional blocking mAbs recognised a unique JAG1 epitope within its DSL domain binding interface with Notch1, effectively blocking ligand-receptor interaction in vitro. Importantly, our mAbs were cross-reactive with rat Jag1 but not the murine orthologue. These were able to inhibit endogenous JAG1-induced signaling in tumor and stromal cells (eg. vascular smooth muscle cells). JAG1 mAb treatment reduced in vitro breast cancer 3D growth, exhibiting reduced expression of important genes such as HES1, IL6 and decreased numbers of cancer stem cells. Treatment did not affect JAG2-induced growth, or signaling mediated by other Notch ligands (eg. DLL4), confirming mAb specificity. Importantly, JAG1 mAb treatment in vivo inhibited Notch signaling and tumor growth in cancer xenograft models in two different host animals. Host body weight, blood tests and histological analysis detected no toxicity.

Conclusions: We generated neutralizing mAbs able to inhibit JAG1-induced signaling both in vitro and in vivo. The ability of JAG1 blockade to impair tumor growth without toxicity indicates it has the potential to make a contribution to the current arsenal of cancer therapeutics. This mAb treatment will be subjected to further in vivo testing, both alone and in combination with other therapeutic approaches, to further expand our knowledge of its clinical potential and mechanism of action.

Funding and conflicts of interest:

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We have filed a patent application, WO/2014/111704, claiming therapeutic use of the JAG1 antibodies.

While FGFR4 and its ligand FGF19 represent a promising target for cancers in which FGF19 is overexpressed or gene amplified, inhibition of this pathway carries a potential toxicity risk due to the role of FGFR4 in bile acid homeostasis. Treatment with an anti-FGF19 antibody caused significant toxicity in cynomolgus monkeys that was attributed to perturbations in the enterohepatic circulation of bile acids. Given that FGF19 signals through other FGFRs besides FGFR4, we hypothesized that the toxicity profile of an anti-FGFR4 antibody might be different than that of an anti-FGF19 antibody. To test this we set out to identify an anti-FGFR4 antibody that inhibits FGF19 binding and displays anti-tumor activity in models overexpressing FGF19, and then determine its toxicity profile.

A phage screening effort yielded several anti-FGFR4 antibodies including H4, a high affinity fully human IgG1. H4 binds to the extracellular domain of FGFR4 (Kd=12 pM) but does not cross react with FGFR1, FGFR2, or FGFR3. Furthermore, H4 efficiently blocks FGF19 binding to FGFR4 (IC50=589 pM) and inhibits FGF19-mediated downstream signaling in Hep3B2 cells. In human liver cancer cells that overexpress FGF19, H4 inhibits proliferation (IC50 250-1,100 nM). Anti-tumor activity was evaluated using cell line-derived xenograft tumors established from the FGF19 overexpressing and gene amplified cell lines HuH-7 and Hep3B2. H4 inhibited tumor growth in these models (T/C of 50% and 8%, respectively), and the antitumor effect was accompanied by alterations in several biomarkers associated with FGFR4 pathway inhibition including increased expression of CYP7A1, the gene encoding the rate-limiting enzyme of bile acid synthesis.

Finally, to assess the potential toxicity of H4 treatment, single dose intravenous toxicity studies were conducted in rats (20, 60, and 200 mg/kg) and cynomolgus monkeys (5, 20, and 100 mg/kg). Although H4 binds with similar affinity to monkey and rat FGFR4, the toxicity profiles were distinct. H4 was well tolerated in rats with no significant findings up to 200 mg/kg. Effects in monkeys included sporadic malformed feces, reduced food intake, elevated serum ALT (up to 44 fold over baseline) and AST activities, and elevated fecal bile acid concentrations at all doses. Hyperplasia of the gall bladder epithelium occurred at 100 mg/kg. There were no microscopic findings in the liver. Qualitatively this toxicity profile is similar to that of the anti-FGF19 antibody, although the maximum tolerated dose (MTD) of H4 in the monkey after a single dose was considered to be >100 mg/kg. These data are relevant for drug development in Oncology in light of the efforts aimed at developing anti-FGFR4 antibodies and small molecule inhibitors specific for FGFR4.

New Molecular Targets

C130 The oncogenic transcription factors TBX2 and TBX3 as attractive targets for anticancer drug development.  Serah Kimani1, Deeya Ballim2, Trevor Sewell1, Edward Sturrock1, Sharon Prince1.  1University of Cape Town, Cape Town, South Africa; 2University of Bath, Bath, United Kingdom.

TBX2 and TBX3 are transcription factors that play critical roles in embryonic development but have no known function in adult tissue, and their overexpression has been associated with a growing list of cancers including melanoma, sarcomas, breast, pancreatic, liver and bladder cancers (1). Previous studies in our laboratory have shown that TBX2 and TBX3 play central but different roles in oncogenesis, where TBX2 functions as a potent growth promoting factor and TBX3 contributes to tumour formation, metastasis and invasion (2-4). Additionally, our data have demonstrated that TBX2 confers resistance to cisplatin by
promoting DNA repair and may also promote oncogenesis through inappropriate survival of cells with damaged DNA (5).

Recent work from our group has provided strong in vitro and in vivo biological evidence that TBX2 and TBX3 are novel targets for development of anti-cancer drugs for cancers where they are overexpressed. Knocking down TBX2 by shRNA induces senescence in advanced melanoma cells, and it results in profound inhibition of proliferation of several metastatic breast cancer cell lines (2,6). Furthermore, depleting TBX2 in metastatic melanoma and cisplatin-resistant breast cancer cells leads to their sensitization to this widely used chemotherapeutic drug, suggesting that targeting TBX2 in combination with currently used therapies could improve their efficacy (5). On the other hand, when TBX3 is depleted by shRNA in some breast cancers and melanomas, cells display reduced anchorage independence, cell migration, colony forming ability and a less aggressive phenotype (2).

We are in the process of exploring ways of identifying potential inhibitors of TBX2/TBX3 oncogenic activity by combining a structure-based approach that focuses on molecular recognition and interaction of TBX2/TBX3 with their target genes, and a medium throughput approach involving screening of small molecule libraries.

References

C131 ‘KRAS addiction’ an artifact of 2D culture? Inhibitors of the mut-KRAS NSCLC 3D growth.

Introduction: KRAS, the predominant form of mutated RAS (mut-KRAS), is found in ~25% of patient tumors across many cancer types and plays a critical role in driving tumor growth and resistance to therapy. We identified CNKSR1 (connector enhancer of kinase suppressor of Ras 1) to be critical for mut-KRAS but not wild type (wt)-KRAS signaling and cell proliferation. Its product CNK1 is a multi-domain organizer protein found as part of the Ras membrane signaling nanocluster where it binds to mut-KRas although not to wt-KRas, and is necessary for mut-KRas cell growth and signaling. We have exploited the pleckstrin homology (PH)-domain of CNK1 as a target for drug discovery to inhibit mut-KRas. To understand the role of CNK1 as a regulator of KRas cell growth, evaluation of siKRAS and siCNK1 on 2D and 3D growth were evaluated. We also studied the effects of KRAS cell growth conditions on response to selective inhibitors the PH-domain of CNK1 identified using a computational modeling approach.
Results: Using a panel of twelve NSCLC lines and siRNA knockdown of KRAS we found that the reported growth addiction of some cancer cell lines for mut-KRAS, and the resistance of others, is most likely an artifact of 2D culture. Mut-KRAS NSCLC cell lines showing addiction in 2D growth did not show addiction in 3D anchorage independent growth in agarose, where all cell lines were more sensitive. Wt-KRAS cell lines were largely unaffected by siKRAS knockdown. Additionally, the CNK1 inhibitor PHT-7390 IC50s for 2D growth inhibition of a panel of 8 mut-KRAS NSCLC lines ranged from 0.60 to 100 µM (ave 18.7 µM) yet was found to be considerably more potent in 3D culture with IC50s from 0.03 to 2.99 µM (ave 0.69 µM). The most pronounced difference was seen in H2009 and Calu1 mut-KRAS cells where PHT-7390 IC50s in 2D were >100 µM, while 0.093 and 0.35 µM in 3D. Wt-KRas NSCLC growth inhibition (3 lines) was largely unaffected by PHT-7390 under either condition with average IC50s of 68 and 69 µM in 2D and 3D conditions, respectively. Target engagement in 2D has shown these CNK1 inhibitors block mut-KRAS signaling but not that of wt-KRAS.

Conclusion: Variable enhanced growth dependence on mut-KRAS (“addiction”) is seen in 2D but not 3D. Potent and specific inhibition of mut-KRAS cell line growth by CNK1 PH-domain inhibitors is considerably more robust in 3D culture suggesting a novel approach to inhibit mut-KRAS effect on cancer growth.

C132 Therapeutic reactivation of PP2A for prostate cancer treatment. Kim McClinch1, Rita Avelar2, David Callejas2, David Kastrinsky1, Michael Ohlmeier1, Stephen Plymate3, Goutham Narla4. 1Icahn School of Medicine, New York, NY; 2Case Western Reserve University, Pepper Pike, OH; 3University of Washington, Seattle, WA.

Several new therapies have recently been approved for patients with castration-resistant prostate cancer (CRPC), however, none are curative and tumors ultimately develop resistance. Advances in the treatment of CRPC require novel approaches and therapies such as those outlined in this study. Most drug development efforts have focused on targeting single oncogenic proteins, an approach limited by the complexity of signaling networks and associated cross talk. Targeting phosphatases, the key negative regulators of signaling proteins, on the other hand, may overcome some of these limitations, particularly if these negative regulators themselves are altered. Through reverse engineering of tricyclic neuroleptic drugs, we have developed a series of small molecule activators of the serine/threonine phosphatase 2A (PP2A), a key negative regulator of numerous oncogenic signaling pathways. PP2A acts as a tumor suppressor and dephosphorylates several critical nodes in prostate cancer pathogenesis including the androgen receptor (AR). Decreased PP2A expression and/or activity have been correlated with castration-resistance in cell culture and human prostate cancer studies. These small molecule activators of PP2A (SMAPs), as represented by TRC-794, TRC-1154, and DT-061, directly bind and activate PP2A and have favorable pharmaceutical properties. In this study we sought to determine the activity of SMAPs in clinically relevant preclinical models of prostate cancer.

Treatment of prostate cancer cell lines with SMAPs resulted in decreased cell viability and colony formation, cell cycle arrest, and an increase in apoptosis. Global Phosphoproteomic analysis of TRC-794 treated prostate cancer cells revealed that the AR and MYC were significantly perturbed in drug treated cells compared to controls which was subsequently confirmed by western blotting. Western blot analysis of prostate cancer cells demonstrated dose-dependent degradation of the AR resulting in PSA reduction and changes in canonical AR target gene expression. In order to investigate whether PP2A was mediating SMAP induced AR degradation, LNCAP cells were stably transduced with the SV40 small t antigen (ST), a potent oncoprotein that perturbs PP2A function. SMAPs were unable to degrade AR in LNCAP cells transduced with ST, suggesting that PP2A mediates SMAP induced AR degradation.
SMAPs were evaluated in vivo in xenograft models representing prostate cancers that are sensitive to conventional therapy and resistant to enzalutamide, the current gold standard, due to overexpression of the AR or expression of androgen receptor splice variants (AR-SV). Single agent treatment with DT-1154 or DT-061 in vivo resulted in either significant tumor growth inhibition or tumor regression and induction of tumor cell apoptosis comparable to enzalutamide. Western blot analysis of the tumors demonstrated that the effects on tumor volume correlated strongly with target engagement as evidenced by significant decreases in PSA and AR expression in vivo. Additionally, these compounds demonstrated favorable pharmacokinetics and showed no overt toxicity. Combined these data highlight the potential for PP2A activation for both the treatment of CRPC and potentially for diverse PP2A inactivated tumor types and diseases.


Chromosomal rearrangements are genomic events that can result in oncogenic gene fusions that are important drivers of tumorogenesis in multiple human cancers. Gene re-arrangements involving RET proto-oncogene were initially reported in papillary thyroid carcinoma. Recent genomic sequencing analysis have identified several rearrangements involving RET gene in other solid cancer types including lung cancers. The fusion protein resulting from the rearrangements usually contain the intact tyrosine kinase domain of RET fused to a partner protein which provides a constitutive dimerization domain. One such rearrangement involved Tripartite repeat motif containing protein 33 (TRIM33) where exons 1-14 of TRIM33 was fused with exon 12-19 of RET which was in frame. Rearrangements involving TRIM33 and RET have been reported in both thyroid cancer and lung cancer. Although these cancers respond to RET inhibitors, the responses are not durable, demonstrating the need for additional treatment approaches to these cancers.

Arsenic, an ancient drug originally used in traditional Chinese medicine, shows remarkable anticancer activity in patients with acute promyelocytic leukemia (APL) and is now part of the standard therapy of this disease. Several studies have given new insight into the mechanism of action and specificity of AS2O3 in treatment of APL. AS2O3 promotes degradation of fusion protein, PML-RARα, a fusion protein containing PML zinc finger protein and retinoic acid receptor alpha. The protein degradation is triggered by arsenic binding to cysteine residues in RBCC domain of PML and PML-RARα. Interestingly PML and TRIM33 both belong to same family of TRIM proteins and have highly conserved RBCC domains, suggesting that arsenic may also induced degradation of TRIM33-fusion proteins. To evaluate the effect of arsenic trioxide on TRIM33-RET fusion proteins, we first synthesized the in-frame fusion gene, and demonstrated that expression of TRIM33-RET fusion protein in primary cell lines leads to transformation. Cells transformed by TRIM33-RET fusion were highly sensitive to arsenic treatment, compared with similar cells expressing other gene fusions. Acute treatment with arsenic induced ubiquitylation and degradation of TRIM33-RET fusion protein. The detailed molecular mechanism of arsenic-induced degradation of the TRIM33-RET fusion and possible role of such treatment in treating cancers with such rearrangements will be presented and discussed. These data suggest that arsenic trioxide, which is now used to target PML-RARα fusion protein in APML, may also be effective in treatment of solid-tumors driven by TRIM33-containing fusion proteins.
C134  Alternative pathways associated with resistance to tyrosine kinase inhibitors in glioblastoma. Hashim M. Aljohani1, Robert F. Koncar1, Ahmed Zarzour2, Byung Sun Park3, So Ha Lee3, El Mustapha Bahassi1. 1University of Cincinnati, Cincinnati, OH; 2Cleveland Clinic Foundation, Cleveland, OH; 3Korea Institute of Science and Technology, Seoul, Korea.

Glioblastoma (GBM) is the most aggressive brain tumor in adults and remains incurable despite multimodal intensive treatment regimens. The majority of GBM tumors show a mutated or overexpressed EGFR. However, the kinase domain mutations that usually correlate with response to the tyrosine kinase inhibitors in other cancers are infrequent in glioblastomas and phase II trials of the tyrosine kinase inhibitor (TKI), gefitinib showed no survival benefit in glioblastoma. Furthermore, tumors treated with the TKIs will inevitably recur, highlighting the need to identify signaling pathways involved in GBM resistance to TKIs. We isolated and developed gefitinib-resistant cell lines and subjected those cell lines to RNA sequencing. By evaluating transcriptomic profiles of the resistant cell lines, we were able to identify several upregulated genes, including a number of other tyrosine kinase receptors such as ROS1, DDR1 and PDGFRA that are known to control pathways downstream of EGFR. The upregulation of these genes was confirmed at the protein level by western blot. Treatment with our highly specific pyrazole ROS1 inhibitor in ROS1-overexpressing cell lines led to sensitization to low doses of gefitinib. Combined treatment with gefitinib and ROS1 inhibitor induced massive cell death by apoptosis following a prolonged cell cycle arrest in S phase. Our current study led to the discovery of alternative pathways used by GBM cells to evade cell death following treatment with gefitinib and identifies new therapeutic targets to prevent GBM cell resistance to the drug.

C136  Identification of selective cancer cytotoxic modulators of phosphodiesterase 3a by predictive chemogenomics. Lucian de Waal1, Timothy A. Lewis2, Matthew G. Rees2, Aviad Tsherniak2, Xiaoyun Wu2, Peter S. Choi1, Lara Gechijian3, Christina Hartigan2, Patrick W. Faloon3, Mark J. Hickey2, Nicola Tolliday2, Steven A. Carr2, Paul A. Clemons2, Benito Munoz2, Bridget K. Wagner2, Alykhan F. Shamji2, Angela N. Koehler2, Monica Schenone2, Alex B. Burgin2, Stuart L. Schreiber2, Heidi Greulich1, Matthew Meyerson1. 1Dana-Farber Cancer Institute, Boston, MA; 2Broad Institute of Harvard and MIT, Cambridge, MA.

High cancer death rates indicate the need for new anti-cancer therapeutic agents. Approaches to discover new cancer drugs include target-based drug discovery and phenotypic screening. Here, we identify phosphodiesterase 3A modulators as cell-selective cancer cytotoxic compounds by phenotypic compound library screening and target deconvolution by predictive chemogenomics. We found that sensitivity to 6-(4-(diethylamino)-3-nitrophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one, or DNMDP, across 766 cancer cell lines correlates with expression of the phosphodiesterase 3A gene, PDE3A. Like DNMDP, a subset of known PDE3A inhibitors kill selected cancer cells while others do not. Furthermore, PDE3A depletion leads to DNMDP resistance. We demonstrate that DNMDP binding to PDE3A promotes an interaction between PDE3A and Schlafen 12 (SLFN12), suggesting a neomorphic activity. Co-expression of SLFN12 with PDE3A correlates with DNMDP sensitivity, while depletion of SLFN12 results in decreased sensitivity to DNMDP. Our results implicate PDE3A modulators as candidate cancer therapeutic agents and demonstrate the power of predictive chemogenomics in small-molecule discovery.

Background: ONC201 is an orally active first-in-class small molecule with strong antitumor activity in preclinical models of advanced cancers. In cancer cell lines and patient samples ONC201 induces activation of the integrated stress response (Ishizawa et al. ASH, 2014) resulting in upregulation of ATF4 and CHOP which in turn regulates several proapoptotic genes namely DR5. ONC201 also causes late-stage inactivation of Akt and ERK, which also results in downstream activation of the apoptotic TRAIL pathway as part of innate immune surveillance (Allen J et al, Sci Trans Med, 2013). Activity of ONC201 is independent of p53 status and mutation agnostic. ONC201 is well tolerated at efficacious doses in animal models, crosses the blood brain barrier, is particularly effective in refractory tumors, depletes cancer stem cells (Ishizawa et al, ASH 2014; Prabhu et al, Blood 2014; Zhao et al, ASCO 2014), and is effective with infrequent dosing preclinically. Based on the compelling efficacy and safety profile of ONC201 as well as the engagement of signaling pathways critical for many cancers, the clinical introduction ONC201 in advanced cancer patients is warranted.

Methods: The first-in-human study of ONC201 (NCT02250781) began in January 2015 as an open-label single-site phase I trial enrolling adult patients with refractory advanced solid tumors and glioblastoma (GBM). Patients with symptomatic brain metastases or prior bevacizumab for treatment of GBM are excluded. The primary endpoint is determination of the recommended phase II dose (RP2D) of single agent ONC201 given orally once every 3 weeks (1 cycle). Secondary endpoints include assessment of pharmacodynamics using select biomarkers for ONC201 (Allen et al, 2015), pharmacokinetics, toxicity, and efficacy. The study employs an accelerated, single patient per cohort, dose escalation design with expansion to a standard 3+3 design if a subject has grade > / = grade 2 toxicity or dose limiting toxicity within cycle 1. The maximum tolerated dose is the highest dose level in which 6 patients have been treated with < / = 1 instance of DLT. Based on pre-clinical models doses for once every 3 week dosing were: dose level (DL) 1-125mg; 2- 250mg; 3- 375mg; 4- 500mg; 5- 626mg.

Results: Single patient dose escalation was completed in cohorts 1 to 4 and 6 patients completed at least 3 weeks of level 5 without any > / = grade 2 drug related toxicity. At DL 6, Mean T1/2 = 7.91 hours (7.01-9.42); Mean Cmax =8.58 ng/mL (3.95 - 19.15) Mean AUC(0-24) = 28057 (15293 - 50597) hr-ng/mL. Ongoing radiographic stable disease is noted in a patient with metastatic castrate resistant prostate cancer.

Conclusions: The RP2D of ONC201 is 625mg on a once every 3 week schedule. Evaluation of PD markers of response and enrollment to a dose expansion safety cohort are ongoing.

Targeting macropinocytosis in pancreatic cancer. James Hunt, Serina Ng, Clifford Whatcott, Daniel D. Von Hoff, Haiyong Han. Translational Genomics Research Institute, Phoenix, AZ.

Pancreatic cancer is the fourth-leading cause of cancer-related deaths in the United States with a 5-year survival rate of <5%. The deadly nature of this disease compounded by a lack of effective treatment options highlights a need for new therapeutics. Past studies have suggested that pancreatic cancer cells acquire the nutrients needed for cell growth through macropinocytosis, an endocytic process by which protrusions from the cell membrane create large vesicles that transport extracellular fluid along with the constituent proteins and lipids into the cell. Additionally, several studies have suggested that macropinocytosis is regulated in part by various genes including p21 protein (Cdc42/Rac)-activated kinase1 (PAK1), ADP-ribosylation factor 6
(ARF6), and sorting nexin 5 (SNX5). In this study, we investigated the effects of knockdown of these three genes on the macropinocytic activity of pancreatic cancer cells. The goal of this study is to assess whether PAK1, ARF6, or SNX5 may serve as potential targets for the suppression of macropinocytosis in pancreatic cancer, which would hamper the cells' uptake of nutrients thus starving the cancer cells and hindering tumor growth. We carried out this study using MIA PaCa-2 pancreatic cancer cells, which are known to exhibit relatively high levels of macropinocytosis. Treatment of cells with siRNA sequences specific to the three genes resulted in a significantly decreased uptake of FITC-labeled dextran as compared to cells treated with non-targeting siRNA and cells that received no treatment, indicating that inhibiting the expression of each of the genes correlates to a reduction in macropinocytic activity. Among the three genes, PAK1 siRNAs exhibited the most significant and consistent inhibitory effect on macropinocytic activity of MIA PaCa-2 cells. We, therefore, further tested the effect of two small-molecule PAK1 inhibitors, namely IPA-3 and PF-03758309, on macropinocytosis. Treatment of cells with the inhibitors resulted in a dose-dependent decrease in the uptake of FITC-labeled dextran and FITC-labeled albumin. The inhibitors also showed potent anti-proliferative activity in pancreatic cancer cell lines. The results of this study indicate that inhibition of PAK1, ARF6, or SNX5 all resulted in reduced macropinocytic activity of pancreatic cancer cells, with PAK1 showing the most promise as a potential therapeutic target for pancreatic cancer.

C139 Selective activation of mutant p53 through acetylation promotes cell death in lung SCC cells. Li Ren Kong1, Chit Fang Cheok2, Boon Cher Goh1. 1Cancer Science Institute Singapore, Singapore, Singapore; 2IFOM-p53Lab, Agency for Science Technology and Research, Singapore, Singapore.

Conventional chemotherapy has been applied indiscriminately and mostly produced unsatisfactory outcome. The development of small molecule inhibitors that target on specific oncogenic signaling has since revolutionized the treatment of cancer. From the perspective of lung cancer, it can be further characterized at the molecular level, such as EGFR, ALK, RET and ROS1, to guide treatment options. However, efforts at defining the molecular underpinnings of squamous cell carcinoma (SCC) of the lung have confirmed a paucity of somatic alterations among the common driver oncogenes, with a high frequency of TP53 mutation, particularly the R158 residue. In this study, we showed that combination of cisplatin and belinostat drastically enhanced cell death in a SCC cell line with loss-of-function p53R158G mutation, and intriguingly, this event is strong associated with p53 acetylation. Despite the emerging evidence demonstrating the gains of oncogenic functions in mutant p53, our data suggest for the first time a restoration of p53-dependent apoptosis through induction of acetylation in SCC cells. To examine this disparity, we generated stable clones expressing p53R158G mutant from Calu-1 cells. Compared with the parental p53-null cells, Calu-1-p53R158G cells were more sensitive to the belinostat/cisplatin combination as indicated by the increase in PARP cleavage and caspase 3 activation. Chip-seq and AmpliSeq were performed to determine the binding regions of p53R158G and its distance from nearest downstream genes, as well as the influence of this mutation and treatment on the transcriptional profiles. Overall, differences were seen in the binding behavior of the mutant protein as compared to the wild-type p53. In some cases, genes found to have differences in binding position, such as CDKN1A but not MDM2, also had significant differences (>1.5 log2 fold change) in gene expression, suggesting a partial and selective activation of p53 pathway. Consistently, substitutions of lysine residues in p53R158G from site-directed mutagenesis effectively rescued apoptosis induced by belinostat/cisplatin combination, further supporting the significance of p53 acetylation in the observed cytotoxicity. Mechanistically, this combination was shown to inhibit the phosphorylation of both HDAC3 and sirtuin-1; whereas silencing of sirtuin-1, but not HDAC3, facilitated the acetylation of p53. Selisistat, a sirtuin-1 specific inhibitor, demonstrated strong synergistic combination with cisplatin in enhancing cytotoxicity and p53 acetylation. Overall, this study provides compelling evidences that inhibition
of sirtuin-1 activity promotes cell death through acetylation of p53R158G. Given the prevalence of p53 mutation in lung SCC, treatment strategy subjecting mutant p53 to therapeutic intervention could be an appealing approach.

Other Topics

C140  Heterogenous approach for heterogenous disease, heterogeneity in cancer genomics and epigenetic approach. M. A. Nezami1, Daniel Stobbe2, Aaron Gould-Simon2, Steven Hager3. 1Pacific Medical Center of Hope, Fresno, CA; 2Valley Metabolic Imaging, Fresno, CA; 3CCARE, Fresno, CA.

Tumor heterogeneity in lung cancer, recently described as a variety of distinct genetic and epigenetic profiles and expressions exists in both forms of inter and intra tumoral patterns resulting in differences in their morphology and biological behavior. Unfortunately, as much as our understanding of new targets in cancer is evolving, we still face a major challenge, we are unable to translate a “target response” to a “clinical response”. The critical question remains as we are not able to pinpoint the driver settings of genes which their alterations have the potential to correlate with survival. This problem is further complicated by implementing cytotoxic and targeted therapies. The first category, cytotoxic therapies, can cause activation of multiresistance genes, cause mutation in the tumor, and further stimulate the tumors stem cells. The second group, targeted therapies, can also cause resistance by inducing mutations in the DNA, as well as enhancing the process called “selective advantage” of the colonies that are not responding to the targeted therapy. This pattern is shown in lung cancer post-targeted-treatment circulatory DNA analysis with a wide range of mutations, confirming the fact that the drug itself has promoted “HETEROGENEITY”. “Sequential tyrosine kinase inhibition” is therefore suggested to reduce the potential of secondary mutations in DNA.

Here we propose a new strategy to use a “heterogenous” approach for a heterogenous tumor. In this approach we have looked in the literature in a systemic review for the mechanisms of resistance in targeted therapies and we have identified epigenetic key regulators that could be targeted. We were especially interested in the hypoxia response targets that were involved with the heterogeneity of the tumor. The more hypoxic tumors, the more they disseminate c DNA. Similarly, the more hypoxic a tumor, the more heterogeneity is seen. We believe that by changing the tumor’s behavior, by epigenetic therapy, we can reduce the tumor’s tendency for “genometastasis” (dissemination of the CTC and c DNA), and hence reduce the tumor’s ability to metastasize.

Results and Discussion: Here we review a case series of patients with advanced metastatic non small cell lung cancer treated with a novel multi targeted epigenetic therapy with promising response.

Conclusion: Our preliminary findings on a subset of advanced heterogenous lung cancer treated with combinational epigenetic method deserves further investigation in a larger scale clinical trial, and can significantly improve the clinical outcome in widely metastatic disease.


Androgen signaling is important for the normal development and function of the prostate as well as for prostate cancer (PCa). We recently found that androgens activated the IRE1α-XBP1 arm of the canonical unfolded protein response (UPR) pathways and simultaneously inhibited PERK-eIF2α signaling. Activation of the IRE1α-XBP1 arm was mediated by direct binding of the androgen receptor (AR) in the vicinity of IRE1, as
well as XBPIs target genes, and increase in their expression. Consistently, AR and IRE1α pathway gene expression are correlated in human PCa samples and XBPIs protein expression is significantly increased in cancer compared to normal prostate. However, the mechanisms behind androgen mediated inhibition of PERK-elf2α signaling are not clear at present. One possible mediator in this regard is P58IPK, an XBPIs target gene that can interact with and inhibit PERK and elf2α phosphorylation. Here, we show that P58IPK expression is increased by androgens in a time-dependent manner and that it plays a pro-survival role in PCa cells. P58IPK knockdown activated PERK expression as well as subsequent elf2α phosphorylation when induced with the UPR activator thapsigargin. These findings suggest that P58IPK mediates, at least in part, the differential androgen effects between the IRE1α and PERK signaling in PCa and may be a potential therapeutic target.


Background: Growth modulation index (GMI) and tumor growth rate (TGR) have been proposed as signs of activity in early phase clinical trials. We explored utility of the efficacy end points in metastatic gastric cancer.

Patients and Methods: We carried out a retrospective study in metastatic gastric cancer patients receiving weekly paclitaxel regimen as second or more line treatment between 2005 and 2013 in Yonsei Cancer Center, and evaluate the association of GMI and TGR with efficacy outcomes respectively. The growth modulation index (GMI) was defined the ratio of time to progression with the nth line (TTPn) of therapy to the TTPn−1 with the n-1th line. TGR was computed during the pretreatment period (reference) and the experimental period. Comparisons used chi-squared and log-rank tests.

Results: A total of 133 patients were enrolled in this study. The median TTPn was 2.7 months, whereas the median TTPn−1 was 5.3 months. Thirty-one patients experienced GMI > 1.2 (23.3%). Median OS was highly correlated with GMI: 6.3 and 7.5 months with GMI ≤ 1.2 and GMI > 1.2 (P = 0.0009). TGR could be evaluated in 52 patients. Among them, 37 patients (71.1%) exhibited a decrease in TGR. The decrease of TGFR was associated with PFS (P = 0.043) and OS (P = 0.045).

Conclusions: GMI and TGR seem to be interesting end points that provides additional information compared with classical criteria. GMI >1.2 and decrease of TGR are associated with significant OS improvement.

Radiotherapeutics

C144 LCL161, a SMAC mimetic, induces preferential radiosensitization in human papillomavirus negative head and neck squamous cell carcinoma through induction of apoptosis. Linlin Yang, Bhavna Kumar, Mitchell Romito, Theodoros N. Teknosa, Amab Chakravarti, Terence M. Williams. The Ohio State University Medical Center, Arthur G. James Comprehensive Cancer Center and Richard J. Solove Research Institute, Columbus, OH.

Background and purpose: Evasion of apoptosis contributes to radioresistance of head and neck squamous cell carcinoma (HNSCC), calling for novel strategies to overcome apoptotic resistance. Second mitochondria-derived activator of caspase (SMAC) - mimetics are a new class of targeted drugs that
specifically induce apoptotic cell death and block pro-survival signalling by antagonizing selected members of the inhibitor of apoptosis protein (IAP) family. The present study was designed to investigate the radiosensitizing effect of a SMAC mimetic, LCL161, in HNSCC and the underlying mechanisms for radiosensitization.

Material and methods: We examined the correlation between mRNA expression of apoptotic pathway associated molecules and HPV status by using The Cancer Genome Atlas Database (TCGA) in which 279 HNSCC tumors are included. Protein expression was investigated in 6 HNSCC cell lines (3 HPV[+], 3 HPV[-]), normal oral epithelial cells (NOE) by immunoblotting, and in primary HNSCC tumors by immunohistochemistry (IHC). Clonogenic survival and Alamar Blue assays were carried out in order to explore the potential of LCL161 as a radiosensitizer in HNSCC cell lines. Cell cycle analysis and Annexin-V assays were performed to investigate mechanisms of radiation-induced tumor cell death. Human tumor xenografts were generated to explore the radiosensitization effect of LCL161 on HPV[-] tumors in vivo.

Results: TCGA database analysis indicated significantly higher mRNA expression of cIAP1 and RELA, lower expression of CASP3, CASP9, and BCL2 in HPV[-] compared with HPV[+] HNSCC tumors. Consistent with these findings, immunoblotting confirmed that protein expression of cIAP1 and p65 encoded by RELA were elevated in HPV[-] HNSCC cells. LCL161 displayed minimal single-agent cytotoxicity with IC50 values between 32 \mu M – 95 \mu M in 6 HNSCC cells. LCL161 is a potent inhibitor of cIAPs, and caused downregulation of cIAP1 at nanomolar concentrations in less than 1 hour, while it showed no obvious effect on NF\kappa B, Stat3, Erk, or Akt activation. Interestingly, we found that LCL161 could induce radiosensitization only in HPV[-] HNSCC cell lines, but not in HPV[+] HNSCC cells. LCL161 mediated radiosensitisation was associated with enhanced activation of caspases-3, -7, -8, -9, and PARP. Blockage of caspase activation via a pan-caspase inhibitor, z-VAD-fmk, largely abrogated LCL161 radiosensitization.

Conclusion: These results suggest that LCL161, a SMAC-mimetic, significantly radiosensitizes a subset of HNSCC tumors, via a mechanism involving caspase activation and apoptosis induction. This agent holds promise for future clinical development as a novel radiosensitizer in the treatment HNSCC HPV[-] tumors.

C145 Targeted radiosensitization of non-small cell lung cancer (NSCLC) through ADAM17 inhibition. Ashish Sharma, Sabine Bender, Oliver Riesterer, Angela Broggini-Tenzer, Martin Pruschy. Laboratory for Applied Radiobiology, Zurich, Switzerland.

Introduction: Radiotherapy is a mainstay for the treatment of locally advanced NSCLC. However, the response rates and clinical outcomes are still disappointing, with the 5-year survival rate being only approximately 15%. Growth and survival of NSCLC cells are often dependent on ectodomain cleavage of membrane-anchored proteins, which is primarily mediated by metalloproteases such as ADAMs (a disintegrin and metalloproteinase). Among all metalloproteases, ADAM17 is actively associated with this process of proteolytic ‘shedding’ and hence the release of growth factors and cytokines regulating cell proliferation and migration. In the current study, we provide novel insights into activation of ADAM17 and subsequent ligand shedding in response to irradiation. We show for the first time that direct targeting of ADAM17 with a clinically relevant inhibitor radiosensitizes lung carcinoma in vitro and in vivo.

Material and methods: Large scale secretome profiling (>300 factors) was performed using antibody arrays for a wide range of secretory factors. Secretion kinetics of selected ADAM17 substrates were determined using ELISA across different established tumor cells and in murine blood serum, derived from irradiated A549 tumor xenograft-carrying mice. Clonogenic survival and xenograft tumor growth delay assays were performed in response to IR in siRNA-targeted tumor cell lines or in combination with small molecular
agents. Tumor proliferation and ADAM17 downstream was evaluated employing immunohistochemistry and ex vivo blood serum analysis.

Results: Based on a large scale secretome screening, we investigated secretion of auto- or paracrine factors in non–small cell lung cancer in response to irradiation and discovered the ADAM17 network as crucial mediator of resistance to IR. Irradiation triggered secretion of multiple ADAM17 substrates as ALCAM and amphiregulin in a similar IR-induced time- and dose-dependent manner across a panel of NSCLC cell lines and from A549-derived tumor xenografts. Irradiation also induced a dose-dependent increase of furin-mediated cleavage of the proform of ADAM17 to active ADAM17, which resulted in enhanced ADAM17 activity in vitro and in vivo. Genetic or pharmacologic targeting of ADAM17 suppressed IR-induced shedding of secreted factors, downregulated ErbB-signaling in target cells and enhanced IR-induced cytotoxicity in vitro. Furthermore, the combined treatment modality of IR with the ADAM17 inhibitor TMI-005 resulted in a supra-additive antitumor response in A549 tumor xenografts. Inhibition of ADAM17 with TMI-005 also strongly reduced immunohistochemical staining for ADAM17 substrates and MIB1, demonstrating an enduring antiproliferative effect and potency of ADAM17 targeting in combination with radiotherapy to overcome treatment resistance.

Conclusions: These findings implicate that radiotherapy significantly activates ADAM17 in non–small cell lung cancer (NSCLC) cells, which results in shedding of multiple survival factors, growth factor pathway activation and contributes to treatment resistance. We provide a sound rationale for repositioning ADAM17 inhibitors as short-term adjuvants to improve the radiotherapy outcome of NSCLC.


Background: In order to understand the changes induced in tumor cells following multi-fraction (MF) radiation therapy, we have previously studied molecular changes using prostate cancer cells and endothelial cells treated in vitro with MF doses of 0.5 Gy/1 Gy x 10 and 2 Gy x 5 and single-dose (SD) of 5 Gy and 10 Gy. The hypothesis being tested is that the response and adaptation to radiation-induced stress will produce a druggable phenotype. This might increase the utility of molecularly targeted therapeutics and also help address tumor cell heterogeneity. The data indicate more genes and pathways are induced by MF compared to SD and that the change in phenotype is more stable following MF. In this report, the focus is on new data from PC-3 cells irradiated in vivo and comparing it to MF and SD in vitro using MF 1 Gy x 10 and SD 10 Gy.

Methods: PC-3 prostate cancer cells were implanted subcutaneously into the lateral aspect of rear leg of nude mice. Mice were divided into three groups (n=3), based on radiation dose/schedule- control, SD, and MF. SD and MF employed similar dose/schedule as used for the in vitro studies, 10 Gy x 1 and 1 Gy x 10 respectively. RNA was isolated 24 h after radiation treatment. mRNA microarray analysis was performed using Agilent Technologies Human Gene Expression 4 x 44 K V2 microarrays. The data was generated and analyzed with GeneSpring® software (Agilent Technologies, Santa Clara, CA) and IPA software (IPA, QIAGEN, Redwood City, CA).

Results: 6,374 genes were significantly altered by MF, with a cohort of genes, based on the > 250 gene ontology categories, involved in DNA response to stimulus, DNA repair, mitosis, cell cycle, and metabolism. In contrast, only 453 genes were significantly altered by SD, with ontological categories associated with cell morphology, assembly and organization such as actin filament-based process, extracellular matrix organization and biogenesis, fibril organization and biogenesis and collagen catabolism. Further
bioinformatics analysis of the gene expression data with IPA, identified multiple pathways with functions correlated with the ontological categories. Significantly altered MF-induced genes are members of pathways which play a central role in DNA replication, recombination, and repair, cell proliferation and metabolism such as HIPPO Signaling, Protein Ubiquitination Pathway, JNK/SAPK Signaling, ERK/MAPK Signaling, G2/M DNA Damage Checkpoint Regulation, ATM Signaling, PI3K/AKT Signaling and Oxidative Phosphorylation. These pathways were uniquely up-regulated by MF treatment, as none of these changes were identified with SD radiation exposure.

Conclusion: Our result show the differential expression pattern between SD and MF, with MF inducing changes in “targetable” molecular pathways.

Ongoing studies: Currently we are in the process of evaluating radiation-induced targets in ATM signaling, DNA damage and repair, and multiple metabolic targets, and their potential for using radiation to prime cells for molecular-targeted drug therapy.

C147 Radiosensitization using gold nanoparticles for effectively targeting molecular pathways in cancer radiation therapy. Rajiv Kumar¹, Jodi Belz¹, Ilanchezhian Shanmugam¹, Wilfred Ngwa², Ross Berbeco², Mike Makrigiorgos², Srinivas Sridhar¹. ¹Northeastern University, Boston, MA; ²Dana-Farber Cancer Institute, Boston, MA.

The use of nanoparticles with high atomic (Z) number have been known to attenuate X-rays and gold nanoparticles (GNPs) have established themselves as potent radiosensitizer to significantly enhance Radiotherapy (RT) treatment. The understanding of the biochemical pathways accompanying GNPs radiation amplification can provide information on the molecular pathways relevant to therapeutic efficacy and thus will pave the way for designing novel therapeutic platforms by targeting these pathways. However, there are significant limitations in the ability to deliver sufficiently potent concentration of GNPs to tumor cells and currently there is very limited understanding of the biology of the radiosensitization using GNPs.

Thus, to improve the efficacy of radiosensitization and subsequently study the impact of radiosensitization on biochemical pathways, we have fabricated a new generation of targeted GNPs formulation. These ultrasmall 2-3 nm gold nanoparticles were functionalized with hetero-bifunctional PEG, imaging agent-AlexaFlour 647 and targeting peptide- RGD. The in vitro studies showed a robust uptake of these GNPs in different cancer cells lines and clonogenic survival assays have demonstrated a 2.8-fold cell kill enhancement with X-rays in HeLa cell line. To evaluate that these GNPs are targeted to the tumor site, we have injected these nanoparticles via different routes in two different tumor animal models and studied the uptake using in vivo optical imaging. The imaging studies showed highly specific tumor uptake in tumor endothelial cells in orthotopic pancreatic tumor mice model injected intravenously with RGD targeted GNPs. The administration of same RGD targeted GNPs formulation via inhalation/instillation (INH) route as opposed to customary intravenous (i.v) route was studied in transgenic lung cancer mice model.

Fluorescence imaging and ex-vivo electron microscopy results showed a significantly higher concentration of GNPs (4.7 times) in the lung tumors of mice when using INH delivery compared to i.v. approach. Further, to understand the radiobiology of GNPs in vitro, we have studied the protein expression of γH2aX and RAD51 using western blots. The studies involving the radiation induced alterations in presence of GNPs to study the impact on cell cycle and DNA damage/repair are currently underway. The studies will help identify the specific pathways that are critical to GNP radiotherapy which can be targeted subsequently to further boost the efficacy of GNP radiotherapy. This work is supported by ARMY/ W81XWH-12-1-0154, NSF DGE 0965843,
RNA and RNA-based Technologies and Therapies

C148  Rapid blood-based test for ALK, RET and ROS1 mRNA fusions and somatic variants in NSCLC. Hestia Mellert, Kristina Koch, Shannon Campbell, Gary Pestano. Biodesix, Inc., Boulder, CO.

Tissue based methods for the detection of ALK fusions include FISH and IHC. Patients with advanced non-small cell lung cancer (NSCLC) may not be good candidates for biopsies and in cases where tissue is obtained, it is not always sufficient for testing. Additionally, determination of the full range of tumor heterogeneity and any impact of molecular variants on therapeutic responses may not be fully captured by the current FISH and IHC tests being conducted for ALK. For these reasons, blood-based testing that uses PCR and NGS methods for detection of mRNA fusion transcripts and somatic variants in circulation are becoming more clinically relevant. Pre-analytic as well as primer design are critical parameters in the development of circulating RNA tests. We have approached the pre-analytic variables by assessing multiple potential sources of fusion RNAs in prospectively-collected plasma samples, including cell-free RNA (cfRNA) and exosomal RNA. Additionally, test development for ALK, RET and ROS1 (ARR) utilized fusion-partner agnostic primers, only requiring knowledge of the fusion kinase sequence. This test technology has previously been shown in FFPE-extracted mRNA specimens to have the unique capability to identify common fusions, including EML4-ALK variants 1 and 3a/b, as well as rare fusions and novel transcript variants. In this study we have extended the capability of this technology to plasma samples from donors previously diagnosed with NSCLC. Data generated during the development of this test involved extraction of RNA from cell lines and donor plasma, processing to cDNA, adaptor ligation and two-step anchored PCR with specificity for human ALK, RET and ROS1. Amplicons were sequenced on a MiSeq (v2 chemistry) and reads were mapped to the human genome and a fusion specific database in order to identify the specific fusion partner(s). Test turn-around time from RNA extraction to results averaged 72 hours. With this test system we evaluated known variant-positive cell lines for EML4-ALK, SLC34A2-ROS1 and CCDC6-RET, as well as remnant prospectively-collected plasma from NSCLC donors (females of less than 65 years of age, and wild-type for circulating EGFR sensitizing and KRAS somatic variants). All cell lines were positive for the respective fusions (lower limit of input RNA at 25ng) and negative in test cross-reactivity experiments (up to 250ng input RNA). The presence of the fusions in the cell lines were independently confirmed with variant-specific qPCR assays. We additionally tested cell-line spiked human donor samples and successfully demonstrated detection of cell-line derived ARR fusions and donor-derived wild-type ALK, RET & ROS1. All human donor samples passed QC measures based on control gene expression. Ongoing experiments are being conducted to further develop this test and evaluate its relevance for routine blood-based clinical laboratory testing using plasma from patients diagnosed with NSCLC.
**Target Identification and Validation**

**C149** Next-generation sequencing of biopsy-free circulating tumor DNA revealed frequent actionable alterations in advanced hepatocellular carcinoma. Sadakatsu Ikeda¹, Kimberly Banks², Richard B. Lanman², Razelle Kurzrock¹. ¹University of California, San Diego, La Jolla, CA; ²Guardant Health, Inc., Redwood City, CA.

Background: Treatment options for advanced hepatocellular carcinoma (HCC) are limited. Tumor biopsy is not routinely performed in HCC and involves risks. As such, genomic analysis of HCC specimens for matched-targeted therapies is not often an option. The utility of cell-free circulating tumor DNA (ctDNA) next-generation sequencing (NGS) to identify somatic alterations and target therapy in HCC patients has not yet been characterized.

Methods: We prospectively evaluated 14 patients with advanced hepatocellular carcinoma (January 2015 - June 2015) using a ctDNA NGS panel of 68 genes (Guardant360). The test sequences complete exons for single nucleotide variants (SNVs) in 68 genes, amplifications of 16 genes, fusion events in ALK/RET/ROS1/NTRK1, and specific insertion/deletion mutations. The mutant allele fraction for detected alterations is calculated relative to wild type in cell-free DNA. The test is sensitive to a single fragment of mutated ctDNA in a 10 ml blood sample, and analytic specificity is >99.9999%. All patients signed informed consent. When appropriate, patients were presented at the UCSD Moores Cancer Center Molecular Tumor Board.

Results: All patients (100%) had somatic alterations (SNVs and/or amplifications) (average = 3.6 alterations per patient [range, 1-8]) with a median mutant allele fraction of 0.29% (range 0.1% - 37.77%). Point mutations were identified in the following genes: TP53 (N = 8 patients), CTNNB1 (4), PTEN (1), CDKN2A (1), ARID1A (1), and MET (1); amplifications were identified in CDK6 (2), EGFR (2), MYC (2), BRAF (1), RAF1 (1), FGFR1 (1), CCNE1 (1), PIK3CA (1), and ERBB2/HER2 (1). Eleven patients (79%) had at least one actionable alteration identified. The patient with a CDKN2A inactivating mutation and a CTNNB1 activating mutation was treated with palbociclib (CDK4/6 inhibitor) and celecoxib (COX-2/Wnt inhibitor); des gamma-carboxy prothrombin level decreased by 84% over 2 months (1410 to 242 ng/ml [normal: 0.0 - 7.4 ng/ml]) (alpha fetoprotein (AFP) low at baseline). Another patient with a PTEN inactivating mutation and a MET activating mutation received sirolimus (mTOR inhibitor) and cabozantinib (MET inhibitor); AFP declined by 63% in one month (8320 ng/ml to 3045 ng/ml [normal: 0-15 ng/ml]). (Imaging studies are pending).

Conclusion: ctDNA profiling is feasible in advanced HCC, and may provide a tissue biopsy-free alternative in these difficult-to-biopsy patients. Two patients who received matched-targeted therapy demonstrated rapid biomarker response. Further study of clinical validity and utility is ongoing.

**C150** High expression of SNAI2 is associated with the emergence of a highly motile fulvestrant-resistant phenotype and is an indicator of poor response to endocrine treatment in estrogen receptor-positive metastatic breast cancer. Carla L. Alves¹, Daniel Elias¹, Maria Lyng¹, Martin Bak², Anne E. Lykkesfeldt³, Henrik J. Ditzel¹. ¹Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark; ²Department of Pathology, Odense University Hospital, Odense, Denmark; ³Breast Cancer Group, Cell Death and Metabolism, Danish Cancer Society Research Center, Copenhagen, Denmark.

Endocrine resistance is a major clinical problem and is associated with the acquisition of aggressive tumor spread and invasion. To investigate the association between endocrine resistance and tumor cell migration and invasion, we evaluated a panel of MCF7-based cell line models resistant to either tamoxifen, aromatase...
inhibitors or fulvestrant. Fulvestrant-resistant cell lines showed a significantly higher migration capacity than the parental fulvestrant-sensitive cell line. Gene expression profiling and data analysis using Ingenuity Pathway Analysis (IPA) of these fulvestrant-resistant/fulvestrant-sensitive cell lines identified potential genes involved in the promotion of invasive and aggressive characteristics in the fulvestrant-resistant phenotype, including SNAI2, a transcription repressor that promotes epithelial-mesenchymal transition and tumor metastasis. The higher gene and protein expression levels of SNAI2 in fulvestrant-resistant cells were confirmed by RT-qPCR, Western blotting and immunocytochemistry. Specific gene silencing using small interfering RNA (siRNA) against SNAI2 decreased the migratory capacity of fulvestrant-resistant cells in vitro. Clinical evaluation of SNAI2 expression in estrogen receptor-positive (ER+) metastatic tumor samples from patients treated with endocrine drugs in the advanced setting (N=86) showed that tumors with higher expression of SNAI2 correlated significantly with shorter progression-free survival (p=0.001). Our results suggest that upregulation of SNAI2 is associated with the emergence of a highly motile fulvestrant-resistant phenotype in vitro and may be a potential therapeutic target in combination with endocrine therapies in tumors expressing high levels of SNAI2.

C151 Systematic discovery of cancer dependencies through deep coverage pshRNA screens. Michael Schlabach¹, Eric Billy², Konstantinos Mavrakis³, Greg Hoffman¹, Tobias Schmelzle⁴, Francesco Hofmann², Nicholas Keen¹, Frank Stegmeier¹, William Sellers¹. Novartis Institute for Biomedical Research, Cambridge, MA; Novartis Institute for Biomedical Research, Basel, Switzerland.

Large scale cancer genome sequencing efforts, such as TCGA, revealed the landscape of genomic alterations across many tumor types, but the functional relevance of these alterations and genetic interdependencies cannot be fully assessed from these datasets. Thus, functional genomic screens, such as pooled shRNA (pshRNA) screens, hold great promise to systematically identify cancer dependencies. However, RNAi discovery screens have been plagued by off target effects causing false positive results and possibly masking on-target effects. In order to address the previous problems with RNAi screens for cancer target discovery, we conducted a large scale pshRNA screening campaign targeting 7500 genes at a depth of 20 shRNAs per gene, across more than 300 cell lines of the cancer cell line encyclopedia (CCLE). The substantial shRNA and cell line depth in this screen significantly increased the robustness of results relative to previous published screens, and allowed the more robust identification of cancer dependencies. Moreover, integration of those growth phenotypes with known features of the cancer cell line encyclopedia (CCLE) enabled the identification of biomarkers (genetic, epigenetic, or proteomic) that correlate with sensitivity, and thus permitted discovery of synthetic lethal relationships. The increased depth provides a robust data set that can be used to find and validate new drug targets as well as infer pathway membership of novel genes by simple phenotypic similarity. The findings of this screen will be presented here.

C152 4-Demethyl-4-cholesteryloxycarbonylpenclozidine (DM-CHOC-PEN) as a cytotoxic L-glutamine agonist in non-small cell lung cancer (NSCLC) involving the CNS. Lee Roy Morgan, Jr.¹, Ed Benes¹, Andrew H. Rodgers¹, Tallat Mahmood², Roy S. Weiner³, Marcus L. Ware⁴, DEKK-TEC, Inc., New Orleans, LA; Detroit Clinical Research Center, Lansing, MI; Tulane University Medical Center, New Orleans, LA; Ochsner Medical Center, New Orleans, LA.

Purpose: 4-Demethyl-4-cholesteryloxycarbonylpenclozidine (DM-CHOC-PEN) is a poly-chlorinated pyridine cholesteryl carbonate whose MOA is via bis-alkylation of DNA @ N7 - guanine and N6 - cytosine. DM-CHOC-PEN has undergone a Phase I study (allowed enrollment of subjects with advanced cancer +/-
CNS involvement) and is being evaluated in a Phase II trial in subjects with advanced cancer involving the brain. Impressive objective responses and improved PFS/overall survival have been observed in subjects with NSCLC involving the CNS. The main aims of this presentation are to document a mechanism for DM-CHOC-PEN's penetration into NSCLC metastases involving the CNS via L-glutamine (GLM) transport.

Methods: Human cancer cell lines [NSCLC - H2087 & H460 and SCLC - SHP-77 & H1417 - obtained from ATCC] were maintained in culture with complete RPMI (5% FBS, pen/strep/Amp. B), supplemented w/ L-glutamine [300 mg/500 mL medium, Sigma] @ 37°C and in moist 5% CO2 conditions. DM-CHOC-PEN was dissolved in tetrahydrofuran, impregnated into ChemoChads® that deliver drug 0.25 - 5 µg/mL in culture. Cells were grown in culture w/wo supplemental GLM for 36 h and then ChemoChads were added. Cytotoxicity was conducted in multi-well plates using the Cytotec® Assay and measuring IC50 values after 24 hr. All assays were conducted in triplicate.

Results: All 4-cell lines grew well in complete RPMI supplemented with L-glutamine. Both SCLC cell lines grew well as clumps of small single cells in suspension, +/- GLM suppl. but were not sensitive to DM-CHOC-PEN, w/ or wo/ GLM supplements. In contrast, both NSCLC cell lines grew as coalescing islands of adhered well-differentiated cells in GLM supplemented medium, but did not grow well in GLM-free medium. Of interest, both NSCLC cell lines were sensitive to DM-CHOC-PEN (IC50 0.25-0.75 µg/mL) w/ GLM-suppl. medium, but were not sensitive (IC50 >5 µg/mL) in GLM-free medium. However, when GLM was added to the GLM-free medium w/ DM-CHOC-PEN, the cells resumed sensitivity to the drug. Similar findings have been observed for 6/7-human NSCLC explants obtained from CNS surgical samples (which will be discussed).

Conclusion: The common structural similarities between DM-CHOC-PEN, GLM and γ-aminobutyric acid (GABA) could allow the sharing of a transport/receptor mechanism into the cerebellum and other CNS safe havens that provide a favorable microenvironment for NSCLC cells to colonize, thrive and grow. The drug when administered IV associates with erythrocytes (~50%), which facilitates its entry into the neoplastic cerebral circulation and now support for its transport into metastatic NSCLC involving the CNS via the GLM transport system is presented. In the absence of GLM, the GLM-transport system shuts down. Of clinical interest is that all subjects with cerebellar NSCLC lesions have responded to DM-CHOC-PEN in the clinical trials with objective responses, improved PFS (6-17+ months) and improved QOL (AACR, Abst. 1161, 2015). The cerebellum offers a unique microenvironment rich in chemical transmitters and amino acids - in particular GLM and γ-aminobutyric acid (GABA). Supported by NCI/SBIR grant - R43/44CA132257.

C153 Investigation of Chk1 as a novel therapeutic target for small cell lung cancer (SCLC). Triparna Sen¹, Pan Tong¹, C. Allison Stewart¹, You Hong-Fan¹, Ying Feng², Jing Wang¹, Lauren A. Byers¹. ¹MD Anderson Cancer Center, Houston, TX; ²BioMarin Pharmaceutical Inc, Novato, CA.

Background: Small cell lung cancer (SCLC) is the most lethal form of lung cancer, accounting for around 15% of lung cancers in the United States, currently having no targeted therapies with established efficacy. Using a high-throughput proteomic screen, we demonstrated overexpression of checkpoint kinase 1 (Chk1) in SCLC. Chk1 being the master regulator of the cell cycle in absence of p53, we hypothesize that Chk1 targeting may be effective SCLC because of near ubiquitous loss of p53. In the present study, we tested the effect of Chk1 targeting by shRNA-mediated knockdown and pharmacologic inhibition in an extensive panel of SCLC cell lines and in animal models.

Experimental procedures: shRNA-mediated knockdown of Chk1 was done in select human SCLC lines and confirmed at the mRNA and protein level. The effect of Chk1 inhibition by LY2606368 alone and in...
combination with cisplatin or the PARP1/2 inhibitor talazoparib was tested in 39 SCLC cell lines. Proliferation was assessed by cell titer glo analysis and effect on cell cycle was tested by flow cytometry. Drug sensitivity (IC50) was then correlated with baseline expression level of >190 total or phosphorylated proteins measured by reverse phase protein array (RPPA) to identify potential predictive biomarkers. In vivo effect of the Chk1 inhibitor with or without cisplatin or talazoparib was tested in syngeneic and xenograft model of SCLC.

Results: shRNA-mediated targeted Chk1 knockdown significantly decreased the proliferation of SCLC lines confirming Chk1 as a viable target (p<0.01). Targeted Chk1 knockdown also increased the expression of pγH2Ax indicating double-stranded DNA damage upon Chk1 inhibition. Chk1 inhibition by LY2606368 had striking single-agent activity in a majority of SCLC lines (median IC50 2-50nM) and the combination of LY2606368 with either cisplatin or talazoparib was additive in a majority of cell lines. Proteomic analysis revealed association between Chk1 inhibitor sensitivity and elevated basal expression of total and phospho cMyc (p=0.01), 14-3-3 protein (p=0.003), pro-apoptotic protein Bax (p<0.05); in contrast, resistance to LY2606368 at multiple doses was correlated to with higher levels of PARP, SGK and p90RSK (p<0.05). In syngeneic in vivo model, single agent LY2606368 was well tolerated and resulted in near-total regression of tumor (>90% reduction in volume) which was durable (ongoing response >50 days in 7/9 animals). The combination with cisplatin or talazoparib also resulted in tumor regression and was significantly more effective than either cisplatin or talazoparib alone. Tumor regression following LY2606368 treatment was also observed in cis-resistant H69 xenograft model of SCLC.

Conclusion: The study established checkpoint kinase 1 (Chk1) targeting as a novel and active therapeutic strategy for SCLC, a disease for which the standard of care has remained unchanged for >30 years. Chk1 knockdown was sufficient to inhibit proliferation in SCLC cell lines and LY2606368, a Chk1 inhibitor in current clinical trials, showed activity as a single agent and in combination with cisplatin and PARP inhibition in vitro and in vivo. TP53-mutant cells (hallmark of SCLC) rely on Chk1 for cell cycle arrest; hence, inhibition of Chk1 in SCLC likely works by driving cells to mitotic catastrophe and apoptosis. These findings support the further investigation of Chk1 targeting and Chk1 predictive biomarkers in SCLC.


Several statistical tools have been developed to identify genes mutated at rates significantly higher than the background, indicative of positive selection, involving large sample cohort studies. However, studies involving relatively smaller sample sizes or single data-type genome wide analysis are inherently restricted due to their limited statistical power to identify low frequency genetic variation. We undertook integrated copy number, mutation and expression analyses of head and neck cancer cell lines assessing multiple levels of biological regulation in individual samples to enrich for driver events affected by two or more alterations. Besides identifying TP53, PTEN, HRAS and MET as major altered HNSCC hallmark genes, this analysis uncovered 34 novel candidate tumor suppressor and oncogenes altered by at least two types of alterations. Of these, we find a driver heterozygous truncating mutation in Nuclear receptor binding protein NRBPI, a pseudokinase, identical to that reported in other cancers and similar to activating mutant alleles in non-human model organisms. In addition to demonstrating the ability of integrated analyses to uncover biologically important genetic variation in relatively small datasets, we report for the first time, mutant NRBPI is oncogenic when ectopically expressed in NIH 3T3 cells. We further show that knockdown of NRBPI in oral carcinoma cell lines bearing such NRBPI mutations inhibits transformation and survival. In over all,
this approach could be of immense value for studies involving fewer or rare clinical specimen that are otherwise inherently restrictive due to the limited statistical power to detect alterations existing at lower frequency.

C155  **Cyclin-dependent kinase 1 (Cdk1) is a promising therapeutic target to overcome ovarian cancer.** Hanbyoul Cho, Wookyeom Yang, Ha-Yeon Shin, Eun-ju Lee, Doo-Byung Chay, Jae-Hoon Kim. Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea.

Purpose: Ovarian cancer shows heterogeneous characteristics which cause chemoresistance and recurrence. The goal of this study was to elucidate characteristics common to various ovarian cancers in order to evaluate their potential for targeted therapy.

Methods: Microarray results for cell lines of serous, mucinous, and brenner types of ovarian cancers were reanalyzed and validated via ovarian cancer cell lines, GEO datasets and TMA blocks. RO-3306 which is an inhibitor of Cdk1 and si-cdk1 was used to measure the growth rate of ovarian cancer cell lines via FACS analysis. In addition, combination treatment with RO-3306 and cisplatin was administered in vitro and in vivo xenograft mouse model.

Results: Microarray reanalysis revealed that expression of Cdk1 and cyclinB1 was higher in 5 types of ovarian cancer cell lines, 4 GEO datasets and ovarian cancer TMA blocks than in HOSE cells. Ovarian cancer TMA particularly showed increased level of cytoplasmic Cdk1 protein, but not in nucleus. When Cdk1 was inhibited by siRNA and a potent Cdk1 inhibitor (RO-3306), growth of ovarian cancer cells decreased, while G2/M arrest or apoptosis increased. In addition, tumor growth was significantly lesser in a xenograft mouse model treated with a combination of RO-3306 and cisplatin than in mice treated with each drug alone.

Conclusions: Cdk1 is a promising gene for targeted anticancer therapy. It is expected that combined treatment with Cdk1 inhibitor and chemotherapeutic agents would maximize the effects of ovarian cancer treatment.

C156  **GPCRs as potential therapeutic targets in pancreatic cancer-associated fibroblasts.** Shu Zhou¹, Sarah Chang¹, Thalia McCann¹, Randall French², Andrew M. Lowy³, Paul A. Insel⁴. ¹Department of Pharmacology UCSD, La Jolla, CA; ²Moores Cancer Center UCSD, La Jolla, CA; ³Moores Cancer Center, Department of Surgery UCSD, La Jolla, CA; ⁴Department of Pharmacology, Department of Medicine UCSD, La Jolla, CA.

Pancreatic ductal adenocarcinoma (PDAC) is characterized by a dense fibrotic stromal matrix, composed of activated fibroblasts (PFs)/stellate cells (PSCs), immune/inflammatory cells and other cell types. This unique tumor microenvironment is increasingly recognized as a key mediator of PDAC progression and drug resistance. Targeting the tumor stroma may thus be a therapeutic approach for PDAC. Pancreatic cancer-associated fibroblasts (CAFs), myofibroblast-like cells that produce extracellular matrix proteins, are responsible for the desmoplasia in PDAC. PSCs and PFs are the key progenitors of CAFs. Blocking the activity of CAFs may be a means to improve the therapy and prognosis of PDAC. We hypothesized that G protein-coupled receptors (GPCRs) expressed by pancreatic CAFs are potential therapeutic targets for PDAC. To begin to test this hypothesis, we used an unbiased GPCRomic array approach to identify and quantify the GPCRs expressed by pancreatic CAFs. We discovered that 104 GPCRs have shared expression in CAFs from the primary tumors of five patients; 35 of those GPCRs had at least a 2-fold increased expression in CAFs compared to both PSCs and PFs. An orphan GPCR that we term Orphan 1 is one of the
most highly up-regulated GPCRs in CAFs. We found that: 1) co-culture of PSCs with a PDAC cell line (ASPC-1) increases expression of fibrotic markers and Orphan 1 GPCR; 2) Transfection of Orphan 1 GPCR in PSCs increases the expression of fibrotic markers; 3) siRNA knockdown of Orphan 1 in CAFs decreases fibrotic marker expression and cytokine secretion. We conclude that GPCRomics can identify GPCRs that regulate pancreatic CAFs. One such GPCR, Orphan 1, contributes to pro-fibrotic activities in CAFs and is a potential therapeutic target to blunt the fibrosis associated with pancreatic cancer.

C157 Syndecan1 is required for oncogenic Kras-driven PDAC tumorigenesis and maintenance. Wantong Yao, Haoqiang Ying, Giulio Draetta. MD Anderson Cancer Center, Houston, TX.

Although the universal presence of KRAS mutations and their critical role in human pancreatic ductal adenocarcinoma (PDAC) designates it as an ideal therapeutic target, oncogenic KRAS (KRAS*) is still regarded as ‘undruggable’ to date. Therefore, to identify therapeutic points of intervention, it is critical to understand the impact of KRAS*-mediated pathways on in vivo tumor pathogenesis. We have recently generated an inducible KrasG12D-driven (iKRAS*) mouse PDAC model and established a critical role for sustained KRAS* activity in tumor maintenance, providing a model to characterize pathways required for KRAS*-dependent tumorigenicity. Cell surface proteins are relatively accessible and can not only provide candidates for biomarker discovery, but also be utilized as therapeutic targets. To characterize the KRAS*-specific organization of cell surface proteins for the development of possible diagnostic or therapeutic tools, we conducted an unbiased surfaceome analyses of tumor cells in the presence and absence of KRAS* signaling using the iKRAS* mouse PDAC model. Syndecans (SDC), a family of heparin sulfate proteoglycans, were identified as candidates whose membrane expression is correlated with KRAS* activity. SDC1 expression is increased in premalignant and malignant pancreatic lesions of primary human PDAC and various KRAS*-driven pancreatic cancer mouse models. In addition, SDC1 expression is specifically upregulated in response to KRAS* induction in acinar-ductal metaplasia (ADM) and early pancreatic intraepithelial neoplasia (PanIN), but not in cerulin-induced chronic pancreatitis. Moreover, SDC1 membrane expression was abolished upon KRAS* extinction in PDAC cells and our data indicated that MAPK pathway, but not PI3K pathway, is important for KRAS*-mediated SDC1 surface localization. To further study the role of SDC1 in KRAS*-driven pancreatic cancers, SDC1 knockout mouse model was generated and crossed with LSL-KrasG12D-PDAC models. Our preliminary data indicated that genetic ablation of SDC1 expression effectively suppressed KRAS*-driven PDAC initiation and progression. The mechanisms of KRAS*-mediated SDC1 membrane localization and its impact on PDAC initiation and progression is being further explored. SDC1 might potentially be the drug target and diagnostic marker for PDAC.

C158 A chemoproteomics strategy for target identification and lead compound optimization using chloroalkane derivatized compounds. Michael Ford¹, Richard Jones¹, Ravi Amunugama¹, Danette Daniels², Rachel Ohana², Sergiy Levin³, Thomas Kirkland², Marjeta Urh⁴, Keith Wood⁵. ¹MS Bioworks, Ann Arbor, MI; ²Promega Corporation, Madison, WI; ³Promega Biosciences LLC, San Luis Obispo, CA.

The Identification and validation of drug targets is an industry wide challenge. There is a very clear and urgent need for technologies that can identify the interaction partners of small molecules. Chemical proteomics is one technology that has attracted attention as a solution to the drug target identification problem. Here we present a new approach utilizing a chloroalkane (CA) moiety capture handle, which can be chemically attached to small molecules to isolate their respective protein partners. In general derivatization of small molecules with the CA moiety does not impact their cell permeability and has limited
impact on potency, allowing for phenotypic assays of the derivatized compound to be performed. The retention of cell permeability also allows for the capture process to be performed from live cells treated with the CA-compound. This process is also compatible with competition assays, which can be used to evaluate and compare other compounds exhibiting a similar mode of action.

Here we present a study using Dasatinib-CA, a modified kinase inhibitor and potent anti-cancer drug which binds to a broad range of kinases. First we performed target enrichment experiments by incubating K562 cells with the modified Dasatinib (Dasatinib-CA). Cells were lysed and the Dasatinib-CA, together with the bound targets, was rapidly captured onto magnetic resin coated with HaloTag. Unmodified compound was used to competitively elute putative interacting proteins. Secondly using the same assay format we evaluated the relative target affinities of Dasatinib-CA versus competing molecules. Competition assays were performed by incubating multiple mixtures of Dasatinib analogues and Dasatinib-CA at varying relative concentrations.

Eluted proteins were processed using SDS-PAGE and in-gel digestion. For target identification experiments peptides were analyzed using label free mass spectrometry. For the competition assays digested material was labeled with Tandem Mass Tags (TMT) 10plex reagents. Peptides were analyzed using nanoscale LC-MS/MS coupled with a Q Exactive mass spectrometer (Thermo). Protein identification and quantitation was performed with MaxQuant (MaxQuant.org) and data validation and visualization was performed using Perseus (Perseus-framework.org).

Using this approach we identified over 30 kinases, including known membrane associated and membrane protein targets. This work presented here highlights a new method for chemical proteomics and demonstrates utility of the platform to enable target identification and to evaluate competitor molecules.

C159  Genome-wide CRISPR-Cas9 screens uncover therapeutic targets and tumor suppressor genes in glioblastoma multiforme.  Chad M. Toledo1, Yu Ding1, Pia Hoellerbauer1, Ryan J. Davis1, Ryan Basom1, Emily J. Girard1, Eunjee Lee2, Philip Corrin1, Hamid Bolouri1, Jerry Davison1, Qing Zhang1, Do-Hyun Nam3, Jeongwu Lee4, Steven M. Pollard5, Jun Zhu2, Jeffery J. Delrow1, Bruce E. Clurman1, James M. Olson1, Patrick J. Paddison1. 1Fred Hutchinson Cancer Research Center, Seattle, WA; 2Icahn Institute of Genomics and Multiscale Biology, Icahn School of Medicine at Mount Sinai, New York, NY; 3Institute for Refractory Cancer Research, Samsung Medical Center, Seoul, Korea; 4Lerner Research Institute, Cleveland Clinic, Cleveland, OH; 5Edinburgh CRUK Cancer Research Centre and MRC Centre for Regenerative Medicine, The University of Edinburgh, Edinburgh, United Kingdom.

Precision oncology is currently based on the notion that genomic analysis of descriptive molecular signatures from patient tumors will lead to actionable therapeutic targets following the patient's arrival into the clinic. However, this approach has failed to deliver new effective treatments for glioblastoma multiforme (GBM), which is the most common and aggressive form of brain cancer; and thus, ~90% of adult GBM patients receiving standard of care therapies continue to die within 2 years of diagnosis. In addition, this approach has neglected the importance for using functional genetics in precision oncology. To identify new therapeutic targets for GBM, we applied functional genetics to perform lethal genome-wide CRISPR-Cas9 knockout screens in patient-derived GBM stem-like cells (GSCs) and also non-transformed human neural stem cells (NSCs), non-neoplastic tissue of origin controls.

Here, we present our latest findings from these screens, which include multiple novel GBM-specific lethal genes that were validated by both in vitro and in vivo preclinical studies. Knockout of GBM-specific lethal genes, including the WEE1-like kinase, PKMYT1/Myt1, lead to lethality in GSCs, but not NSCs. Focused
mechanistic studies revealed that PKMYT1 acts redundantly with WEE1 to phosphorylate CDK1-Y15 and to promote timely completion of mitosis in NSCs, but that this redundancy is lost in most GBM isolates and in NSCs harboring activated alleles of EGFR and AKT1, which are commonly altered signaling pathways in GBM. Moreover, PKMYT1 depletion in GSCs and genetically altered NSCs requiring PKMYT1 lead to cytokinesis failure and cell death during mitosis.

In addition to lethal genes, genes promoting in vitro expansion of NSCs upon knockout were examined. For this category, we validated multiple genes that are candidate tumor suppressors and involved in: the negative regulation of Hippo signaling, TP53 signaling, epigenetic regulation, promoting neural development, and other cellular functions. Knockout of these genes caused shortened cell cycle transit times and drastic growth advantages in NSCs, and in the case of CREBBP knockout, caused precocious entry into S-phase and deregulation of cell cycle gene expression. The identification of these potential tumor suppressors here reveal new genetic drivers in glioma/GBM, which contributes to a growing body of work that will help redefine GBM gene signatures.

Furthermore, we found that the molecular signatures of pathways and genes commonly altered in GBM are not ideal GBM therapeutic targets since most of these targets failed to score as GBM-specific lethal hits in our knockout screens and likely are non-essential or essential in both the GSCs and NSCs. Nonetheless, these common GBM alterations give rise to cancer-specific vulnerabilities, which then lead to genes, such as PKMYT1, that are required to overcome functional impairments in cancer cells. Taken together, we demonstrated here that genomics and functional genetics are equally important for precision oncology, as the combination of both tools can identify genetically altered genes, cancer-specific therapeutic targets, and tumor suppressors. These results are part of a rapidly growing body of work by the science community that will one day allow oncologists to tailor therapies for each patient based upon his or her tumor genetic profile and characteristics.

CI60 An RNAi kinome screen identifies YES1 as a potential target for glioblastoma. Surbhi Goel-Bhattacharya, Sejuti Sengupta, Brent H. Cochran. Tufts University, Boston, MA.

Glioblastoma stem like cells (GSC) are known to have gene expression patterns and growth phenotypes more similar to the tumor of origin than do traditional glioma cell lines grown in serum. They also exhibit increased resistance to drugs and radiation and are likely to be responsible for tumor recurrence following therapy. As a result, it is important to identify therapeutic targets for these cells. Toward this end, we have conducted RNAi kinome screens in GSCs to identify genes that are required for their growth and survival. In addition, glioblastomas (GBM) often have significant regions of hypoxia and GSCs tend to be resistant to hypoxia. We have therefore conducted shRNA screens in both normoxic and hypoxic conditions and screened three independent patient derived GSC lines. Of 501 kinases screened, 8-11% were scored as hits with at least 2 shRNAs per kinase in each cell line, but only ~2 % were common hits for all the cell lines in both oxygen conditions. The majority of the hits were shared in both oxygen conditions, but about one sixth were specific to hypoxia in each of the cell lines. However, none of the hypoxia specific hits were common to all three cell lines. Several genes well known to have a role in GBM such as EGFR, CDK4 and KDR were scored as common hits. The non-receptor associated tyrosine kinase YES1 was scored as a novel hit common to all the three cell lines in both the oxygen conditions. YES1 is overexpressed in GBM and several other solid tumors. Depletion of YES1 expression by shRNA and CRISPR was found to be strongly growth inhibitory in GSCs suggesting that it could be a therapeutic target for GBM. Ongoing experiments include studying the mechanism of YES1 associated cell death.
C161  **CRISPR/Cas9 genome-wide gRNA library screening platform.** Donato Tedesco, Paul Diehl, Mikhail Makhanov, Sylvain Baron, Dmitry Suchkov, Alex Chenchik. Cellecta, Inc., Mountain View, CA.

CRISPR/Cas9 gene knock-out technology can be used as a powerful tool for large-scale functional genomic analysis in mammalian cells. With the goal to establish a cost-effective functional genomics platform for the discovery of therapeutic targets, we developed a highly functional high throughput lentiviral gRNA/CRISPR screening platform, enabling scientists to perform pooled format genome-wide CRISPR/Cas9 genetic screens. Three 55K gRNA libraries were designed to cover the whole protein-encoding human genome with a redundancy of 8 gRNAs per gene. As supporting tools, we developed protocols, reagents, and software tools for hit validation and target prioritization.

Here we show the results of parallel genetic screens in a pair of isogenic CML cell lines (CML-R and CML-P), where the performance of CRISPR/Cas9 and RNAi screening platforms was compared for each cell line.

C162  **A medium-throughput single cell CRISPR-Cas9 assay to assess gene essentiality.** Alexandra R. Grassian1, Tim Scales2, Sarah K. Knutson1, Nicola J. McCarthy1, Chris E. Lowe2, Jon D. Moore2, Robert A. Copeland1, Heike Keilhack1, Jesse J. Smith1, Julie A. Wickendon2, Scott Ribich1. 1Epizyme, Inc, Cambridge, MA; 2Horizon Discovery, Cambridge, United Kingdom.

Target selection for oncology and other indications is a critical step in the successful development of therapeutics, however it remains one of the most challenging areas of drug discovery. In fact, up to two-thirds of oncology relevant targets reported in literature have not been confirmed on follow-up studies, indicating that target validation in oncology is especially challenging. Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 gene editing of specific loci offers an alternative method to RNA interference and complements small molecule inhibitors for determining whether or not a cell line is dependent on a specific gene product for proliferation and/or survival. Importantly, CRISPR-Cas9 may be advantageous for some studies as it offers efficient and specific gene knock-out leading to complete loss of protein function. This may be especially useful for some target classes, including epigenetic targets, which appear to require near complete loss of protein function to observe phenotypes. In our initial studies using CRISPR-Cas9 to verify the essential nature of EZH2 (Enhancer of Zest 2) expression for the proliferation of SMARCB1/SNF5/INI1 mutant malignant rhabdoid tumor cell lines, we observed that the initial reduction in proliferation was lost over time. We hypothesized that in the few cells that retain proliferative capacity at least one allele of EZH2 remains functional, and this hypothesis suggests that carrying out CRISPR-Cas9 studies for individual target genes without analyzing single cell clones could produce misleading results. To verify this, we developed a medium throughput assay to analyze 10s-100s of single cell clones for target gene disruption using CRISPR-Cas9 gene knockout, followed by a restriction digest and fluorescent undigested fragment length analysis to successfully assess EZH2 allele status. Significantly, these data support our hypothesis that retention of one functional copy of EZH2 is required for the proliferation of EZH2-dependent cell lines and that this can be rapidly assessed by our assay. Thus, the assessment of zygosity of the gene of interest can be evaluated in a medium-throughput manner in single cell clones. The assay is thereby able to unambiguously indicate whether or not a specific gene is essential for survival and/or proliferation in a given cell line, and offers a unique approach for target validation using gene editing. Importantly, this approach should be applicable to any target of interest that is expected to affect cell proliferation or survival. Such data can aid in the development of more robust cancer therapeutics by increasing confidence in target selection.
Therapeutic Agents: Biological

C163  Understanding the mechanism for synergistic anti-tumor activity of a mesothelin-targeted immunotoxin and taxanes. Christine Campo Alewine, Emily Kolyvas, Ira Pastan. National Cancer Institute, Bethesda, MD.

Recombinant immunotoxins (RITs) are antibody-based therapies that carry a bacterial toxin payload. RG7787 is a next generation RIT that targets the cancer antigen mesothelin (MSLN) to deliver a recombinantly engineered variant of Pseudomonas exotoxin A (PE) into the cell cytosol. PE irreversibly modifies elongation factor-2 (EF-2) by catalyzing addition of an ADP-ribose moiety. This causes EF-2 inactivation, inhibition of new protein synthesis, and a drop in the short-lived pro-survival factor mcl-1, triggering apoptosis. Combination of RG7787 with paclitaxel or nab-paclitaxel results in boosted anti-tumor efficacy in several mouse models. In the KLM1 pancreatic cancer xenograft model, either drug alone halts tumor growth while the combination produces complete regressions. Here, we show that enhanced efficacy is also seen using an identically structured RIT that targets the transferrin receptor instead of mesothelin (LMB-74), but is not seen when RIT is combined with gemcitabine, suggesting that the effect is specific to both taxane and PE. Further, we have found that the interaction of RG7787 and taxane can be modeled in vitro. Under these conditions, the interaction is dependent on the dose of RG7787, and cannot be replicated by a chemical inhibitor of protein synthesis. Co-treatment with taxane does not affect the depth, rate of onset or recovery of cells from RG7787-mediated protein synthesis inhibition. Tumor cell killing occurs over a delayed time course and is not associated with an early drop in mcl-1 levels as is seen when using a cytotoxic dose of RG7787 alone. Taken together, these data suggest that PE treatment sensitizes cancer cells to taxane-mediated killing.


Both Notch/Delta-like ligand 4 (DLL4) and vascular endothelial growth factor (VEGF) pathways play a critical role in angiogenesis and tumor growth. Due to differential regulatory effects of VEGF and DLL4 on the vasculature, blockade of DLL4 or VEGF signaling inhibits tumor growth by distinct mechanisms: anti-DLL4 treatment induces an abnormal increase of poorly perfused blood vessels, which results in nonproductive angiogenesis unable to support tumor growth, whereas anti-VEGF therapy significantly decreases vasculature reducing the blood supply to tumors. In addition, DLL4-Notch signaling plays a key role in the maintenance of cancer stem cells. We have recently developed a bispecific monoclonal antibody that targets both human DLL4 and human VEGF (OMP-305B83). In vitro, this antibody exhibited low nanomolar binding affinity to hVEGF and hDLL4, and reduced human endothelial cell proliferation induced by VEGF. The bispecific antibody demonstrated significant in vivo anti-tumor efficacy in various solid tumors, induced tumor regression, decreased the frequency of tumor initiating cells, and delayed tumor recurrence following termination of chemotherapy. Analysis of tumor vasculature after treatment with anti-DLL4/VEGF revealed inhibition of vascular gene expression and endothelial cell proliferation, indicating that the anti-VEGF effect on the vasculature is dominant over the anti-DLL4 effect. Notably, at doses where both anti-DLL4 and anti-VEGF alone produces suboptimal anti-tumor effect, dual targeting resulted in additive tumor growth inhibition. The combination of anti-DLL4 and anti-VEGF resulted in broad spectrum efficacy in many different solid tumor types including breast, colon, ovarian and pancreatic tumors. Notably, serial transplantation studies indicated that the anti-cancer stem cell activity of anti-DLL4 was retained with the
bispecific. In safety studies, OMP-305B83 demonstrated an improved cardiac profile in cynomolgus monkeys compared to anti-DLL4 with reduction of endothelial hyperplasia and suppression of vascular-related gene upregulation in the heart. These results indicate that our bispecific anti-DLL4/VEGF is broadly efficacious and may be useful for treatment of a variety of tumor types. We are currently enrolling patients with advanced refractory solid tumors in a Phase 1a clinical trial.


Antibody drug conjugates (ADCs) have shown their greatest clinical utility when targeting antigens expressed at very high levels on cancer cells that have coincidentally lower expression in normal tissues. This is exemplified by the approvals of trastuzumab emtansine for her2neu 3+ breast cancer and brentuximab vedotin for Hodgkins Disease and Anaplastic large-cell lymphoma. Both drugs are approved for subsets of specific cancer types where target antigen expression is particularly high relative to expression in normal tissues. There are other cell surface antigens that are highly expressed on cancer cells and normal tissues, but the utility of such antigens as ADC targets is restricted by their corresponding expression in normal tissues. One such target is CD166 (ALCAM), which shows 3+ expression by IHC in most donors of multiple cancer types, e.g., ca. 70% prevalence in breast, prostate, and lung cancers but also expression in multiple normal tissues including lung, GI tissues, and liver. Thus CD166 has not been progressed as a target for ADCs.

Probody™ therapeutics are fully recombinant antibody prodrugs that are converted to active antibodies by tumor-associated proteases. Preclinical in vivo studies show that Probody therapeutics remain substantially inactive in normal tissues and in circulation. As such, Probody drug conjugates (PDCs), unlike ADCs, enable targeting of high expression tumor targets that are also expressed in normal tissues. We have developed an anti-CD166 Probody therapeutic selected for specific binding, internalization, and cross reactivity to cynomolgus macaque as a species for toxicology assessments. This therapeutic has been conjugated to spdb-DM4 and tested in preclinical models of efficacy and safety. Treatment with the PDC has led to complete regressions in models of lung and breast cancer at therapeutically relevant doses. These same doses were assessed for safety in cynomolgus monkeys. The safety and efficacy profiles for the anti-CD166 PDC are supportive of progression to clinical development of this anti-CD166 Probody drug conjugate.

C166 Combining an anti-Trop-2 antibody-SN-38 conjugate (sacituzumab govitecan) with microtubule inhibitors (paclitaxel and eribulin mesylate) or PARP inhibitor (olaparib) significantly improves therapeutic outcome in experimental triple-negative breast cancer (TNBC). Thomas M. Cardillo, Serengulam V. Govindan, Maria Zalath, Roberto Arrojo, Robert M. Sharkey, David M. Goldenberg. Immunomedics, Inc., Morris Plains, NJ.

Purpose: Determine whether combining sacituzumab govitecan (IMMU-132), an anti-Trop-2/SN-38 antibody-drug conjugate, with microtubule inhibitors (paclitaxel or eribulin mesylate) or a poly(adenosine diphosphoribose) polymerase (PARP) inhibitor (olaparib) in mice bearing human TNBC xenografts improves anti-tumor effects.
Experimental Procedures: Mice bearing human TNBC xenografts (MDA-MB-468 or HCC1806; ~0.3 cm³) were treated with the maximum tolerated dose of paclitaxel (15 mg/kg weekly x 5 wks) and IMMU-132 at either 10 mg/kg or 12.5 mg/kg on days 1, 8, 22, and 29. Mice bearing HCC1806 tumors (~0.28 cm³) were treated for 2 cycles with IMMU-132 (12.5 mg/kg) and 0.5 mg/kg of eribulin mesylate (equivalent to human dose of 1.4 mg/m²) weekly for 2 weeks on a 21-day cycle. Studies examining PARP inhibition used mice bearing MDA-MB-468 tumors (~0.32 cm³) treated with olaparib (50 mg/kg, qdx5d, x 4 wks; 33% of human dose equaling 800 mg daily) and IMMU-132 (10 mg/kg, twice weekly x 4 wks). The primary endpoint was the median survival time (MST), defined as the time for tumors to progress to 1.0 cm³.

Results: Mice with MDA-MB-468 tumors given the combination of IMMU-132 and paclitaxel exhibited superior anti-tumor effects, with >11-fold tumor shrinkage, in comparison to 1.4-fold shrinkage in the IMMU-132 group alone (P=0.0003; area under the curve, AUC) or 11.4-fold increase in tumor size in mice treated with paclitaxel alone (P<0.0001; AUC). In the rapidly-progressing HCC1806 xenografts, the combination improved MST to 38 days from 17.5 and 17.0 days for paclitaxel and IMMU-132, respectively (P<0.0015; log-rank). Mice treated with the combination of IMMU-132 plus eribulin mesylate exhibited a significantly greater anti-tumor response than all other monotherapy groups (P<0.0432; paired t-test). This resulted in a significant survival benefit for the combination (MST=23 days) when compared to eribulin or IMMU-132 monotherapy (MST=18 and 14 days, respectively; P<0.0044; log-rank). Likewise, combining IMMU-132 therapy with olaparib was superior to single agent therapy in mice bearing MDA-MB-468 tumors (P<0.0032; AUC). All the IMMU-132 combination treatments were well-tolerated.

Conclusions: IMMU-132 is a humanized anti-Trop-2 antibody conjugated to SN-38, the active metabolite of irinotecan, a topoisomerase I inhibitor. Clinically, IMMU-132 has shown manageable toxicity and encouraging responses in patients with relapsed/refractory TNBC (ClinicalTrials.gov, NCT01631552). Since preclinical studies indicate IMMU-132 can be combined with two different microtubule-inhibitors or a PARP-inhibitor with significantly enhanced anti-tumor activity, these data provide a rationale for future clinical evaluation of IMMU-132 in combination with these and other chemotherapeutics that likewise target cell division through microtubule inhibition or DNA-repair mechanisms in patients with TNBC.

C167 KTN0158, a humanized anti-KIT monoclonal antibody, demonstrates antitumor activity in dogs with mast cell tumors. Cheryl London1, Sarah Rippy1, Heather Gardner1, William Kisselberth1, Gerald Post2, Neal Janson3, Linda Crew3, Theresa LaVallee3, Richard Gedrich3. 1The Ohio State University, Columbus, OH; 2The Veterinary Cancer Center, Norwalk, CT; 3Kolltan Pharmaceuticals, Inc., New Haven, CT.

Background: KTN0158 is a novel humanized anti-KIT IgG1 monoclonal antibody that binds canine, feline, non-human primate and human KIT with high affinity. KTN0158 potently inhibits wild-type and some oncogenic variants of KIT in vitro and modulates canine mast cell function and survival in vivo. A clinical trial was conducted in dogs with spontaneous mast cell tumors (MCT) where KIT is known to be widely expressed and where activating mutations in the form of internal tandem duplications (ITDs) in exon 11 are found in 30% of tumors. The purpose of this study was to evaluate the safety, biologic activity and pharmacokinetic/pharmacodynamic (PK/PD) profile associated with KTN0158 administration in dogs with MCT.

Methods: Twelve dogs with measurable MCT were enrolled into this open-label phase 1 dose-escalating clinical trial. Three dose levels and 2 schedules were evaluated (10 or 30 mg/kg given on Day 0 only; 1 or 10 mg/kg given on Days 0 and 21). Serial tumor biopsies and blood samples for PK and PD analysis were collected pre- and post-treatment throughout the study. Dogs were assessed weekly with physical
examination and standard laboratory tests (serum chemistries, hematology profiles, and urinalyses) for clinical toxicities and response. Adverse events (AEs) were recorded and graded according to published common terminology criteria for AEs in dogs. Determination of antitumor efficacy was based on objective tumor assessments made according to established RECIST criteria for solid tumors in dogs.

Results: Of the 12 dogs entered in this study, 3 had tumors with exon 11 ITDs and 6 had evidence of metastasis to the local lymph node. All dogs treated with a single dose of KTN0158 at 10 or 30 mg/kg or two doses of KTN0158 at 1 or 10 mg/kg experienced clinical benefit. Partial responses were observed in 5 dogs (n=1 KIT ITD) and stable disease was observed in 7 dogs (n=2 KIT ITD). Upon study completion, 11 dogs underwent surgical removal of the MCT with or without extirpation of the draining lymph node. Histopathology failed to identify neoplastic mast cells in tumor samples from 2 dogs (patients 2 and 4) and 3 draining lymph nodes from 3 dogs (patients 2, 3, and 11) classified as metastatic at study entry. The most common adverse events associated with KTN0158 administration were reversible dose-dependent hematologic changes including anemia, neutropenia, and thrombocytopenia, generally occurring 7-14 days post-treatment. Doses of 30 mg/kg KTN0158 resulted in clinically significant toxicities not observed at other dosing levels, including evidence of edema/erythema, grade 4 neutropenia, and grade 3 thrombocytopenia. Evaluation of PK and PD biomarkers is ongoing.

Conclusions: KTN0158 given at 1 and 10 mg/kg demonstrated an acceptable adverse event profile and single agent biologic activity in a relevant spontaneous large animal model of KIT driven malignancy (MCT). The objective responses observed in tumors with and without activating KIT mutation suggest that KTN0158 may provide clinical benefit to patients with GIST and other KIT-driven tumors and human clinical studies are planned.

A two stage prospective clinical trial with irofulven treatment targeting a selected subgroup of castration- and docetaxel resistant prostate cancer patients. Steen Knudsen1, Thomas Jensen2, Anker Hansen3, Ulla Buhl3, Annie Rasmussen3, Bruce Pratt4, Arun Asaithambi4, Nils Brünner5, Peter Buhl Jensen5. 1Medical Prognosis Institute A/S and Oncology Venture AB, Hoersholm and Flagstad, Denmark; 2Medical Prognosis Institute A/S, Hoersholm, Denmark; 3Oncology Venture AB and Medical Prognosis Institute A/S, Hoersholm, Denmark; 4Lanthern Pharma, Dallas, TX; 5University of Copenhagen and Oncology Venture AB, Copenhagen and Hoersholm, Denmark.

Irofulven, a DNA damaging semi-synthetic analog of Illudin S (phytotoxin from Omphalotus illudins, jack-o’-lantern mushroom) which in the body is activated by prostaglandin reductase, has shown promising clinical activity in a range of cancer forms but the objective response rates were too low to justify further clinical development. Of particular interest is that irofulven may not be a substrate of the mdr-1 drug efflux pump and thereby potentially active in prostate cancer patients with acquired resistance to docetaxel. We have now developed an irofulven responsive predictor which is based on gene expression data by comparing associations between gene expression profiles and growth inhibition in a panel of cell lines treated with irofulven. A second step has included filtering the identified gene expression profile against mRNA expression from a collection of 3200 human tumors. Only genes being differentially expressed in the clinical tumor material were retained in the model. A total of 205 mRNA’s were selected for the final irofulven responsive profile. The profile can be converted to a single score of predicted irofulven responsiveness. We are now initiating a prospective clinical trial (Simon’s two stage design) in selected patients with castration- and docetaxel resistant prostate cancer and with a favorable irofulven responsive profile. We are screening 600 prostate cancer samples (FFPE tissue) in order to select the 10% of patients with the highest likelihood
C169  ER maleate is a novel anticancer agent in oral cancer cells: Implications for cancer therapy. Guodong Fu, Sr.. Mount Sinai Hospital, Toronto, ON, Canada.

ER maleate was investigated as a novel anticancer agent for oral squamous cell carcinoma (OSCC), the most common head and neck squamous cell carcinoma (HNSCC). We identified its putative molecular targets, demonstrated their clinical relevance, and showed its chemosensitization potential for platinum drugs to aid in HNSCC management. Biologic effects of ER maleate were determined on cell proliferation, spheroid/colony formation, caspase activity, cell migration and invasion in vitro and using oral tumor xenografts in vivo. mRNA profiling, real time PCR and western blot suggested ER maleate modulated the expression of polo-like kinase 1 (PLK1) and spleen tyrosine kinase (Syk). Their clinical significance was determined in oral SCC patients by immunohistochemistry and correlated with prognosis by Kaplan-Meier survival analysis. ER maleate induced cell apoptosis, inhibited proliferation, colony formation, migration and invasion in oral cancer cells. Imagestream analysis revealed cell cycle arrest in G2/M phase and increased polyploidy, unravelling deregulation of cell division and cell death. Mechanistically, ER maleate decreased expression of PLK1 and Syk, induced cleavage of PARP, caspase 9 and caspase 3 and increased chemosensitivity to carboplatin; significantly suppressed tumor growth and increased antitumor activity of carboplatin in tumor xenografts. ER maleate treated tumor xenografts showed reduced PLK1 and Syk expression; clinical investigations revealed overexpression of PLK1 and Syk in oral SCC patients that correlated with disease prognosis. In conclusion, our in vitro and in vivo findings provide a strong rationale for pre-clinical efficacy of ER maleate as a novel anti-cancer agent and chemosensitizer of platinum drugs for OSCC.


Background: IMGN853 is a folate receptor α (FRα)-binding antibody-drug conjugate (ADC) that utilizes the potent tubulin-targeting maytansinoid, DM4, as its cytotoxic agent. FRα is highly expressed in many solid tumors, particularly epithelial ovarian cancer (EOC), endometrial cancer and non-small cell lung adenocarcinoma. IMGN853 is currently being evaluated as monotherapy in FRα-positive solid tumors in a Phase 1 trial (NCT01609556), with encouraging results recently reported in 17 evaluable patients treated at 6.0 mg/kg adjusted ideal body weight (AIBW) with platinum-resistant EOC (Moore K et al, 2015).

Methods: EOC cell line xenograft models plus EOC patient derived xenograft (PDX) models that had FRα expression representative of patients enrolled in the Phase 1 trial were used to assess IMGN853 single agent and combination therapy activity. Anti-cancer therapies used in EOC were assessed. Results from studies with bevacizumab (Bev), carboplatin and pegylated liposomal doxorubicin (PLD) are reported herein.

Results: IMGN853 plus Bev was assessed in multiple models including OV90 and IGROV-1 EOC cell line xenografts and a platinum-resistant EOC PDX model, and was consistently more active than either agent alone. In most studies, monotherapy IMGN853 or Bev was active with few partial or complete regressions. In contrast, combination IMGN853 + Bev was highly active, with a majority of the animals having partial or complete tumor regression. The combination activity was substantially more than additive and studies to
understand the mechanism(s) responsible for the enhanced activity are ongoing. Combination carboplatin + IMGN853 was more active than carboplatin + paclitaxel in OV90 EOC xenografts. The addition of Bev to carboplatin + paclitaxel enhanced activity compared to carboplatin + paclitaxel. Carboplatin + IMGN853 was more efficacious than the triple combination of carboplatin + paclitaxel + Bev. Carboplatin + IMGN853 + Bev was the most active combination with all mice having tumors that completely regressed. Finally, the combination of PLD and IMGN853 was highly active in a platinum-resistant EOC PDX model, and much more active than PLD or IMGN853 alone. All combinations with IMGN853 described above were well tolerated.

Conclusion: Combination therapy efficacy of IMGN853 with Bev was substantially more than additive in multiple models of platinum resistant EOC. Combination IMGN853 + PLD is more efficacious than either monotherapy and combination IMGN853 + carboplatin is more efficacious than carboplatin + paclitaxel in the models studied. Addition of Bev to the carboplatin + IMGN853 combination further enhanced activity. Studies to understand the mechanism(s) responsible for the enhanced combination activity are under way. The efficacy observed in these models suggests that IMGN853 in combination with PLD, or Bev and/or carboplatin may be promising regimens to evaluate in clinical trials of EOC both in the relapsed and upfront settings. A phaseIb clinical study assessing doublet combinations of IMGN853 with PLD, Bev and carboplatin in relapsed EOC is planned for 2015.

C171 Human anti-nucleolin recombinant immunoagents as new potential tools for melanoma treatment. Ashley Braddom1, Timothy Richmond1, Tyler Sheetz1, Erika Reese1, Anna Tessari1, Kathleen Tober1, Christin E. Burd1, Claudia De Lorenzo2, Edward W. Martin, Jr.1, Vincenzo Coppola1, Michael F. Tweedle1, Tatiana Oberyszyn1, Carlo M. Croce1, Dario Palmieri1.1The Ohio State University, Columbus, OH; 2Universita’ degli Studi di Napoli, Napoli, Italy.

Immunotherapy and immune-based anti-cancer molecules represent a valid strategy to fight cancer. However, the choice of tumor-specific surface molecules for the selective targeting of cancer cells still represents a critical step in the study design for the development of new therapeutic approaches. Notably, the development of phage-display technology for the selection of fully human single chain antibody fragments (scFvs) and complete antibodies directed toward tumor-associated antigens has represented a significant advancement for immunotherapy.

Nucleolin (NCL) is one of the most abundant non-ribosomal proteins in the nucleolus. NCL is frequently up-regulated in cancer and in cancer-associated endothelial cells compared to normal tissues, where it is also present on the cell surface. Altered NCL expression and localization results in oncogenic effects such as stabilization of oncogenic mRNAs and microRNAs (miRNAs). Particularly, we demonstrated that NCL enhances the maturation of specific miRNAs (including miR-21, miR-221 and miR-222) causally involved in cancer pathogenesis, aggressiveness, metastatic potential and resistance to several anti-neoplastic treatments.

Because of its oncogenic role and specific expression on cancer cell surface, NCL represents an attractive target for anti-neoplastic therapies. To produce a new anti-NCL molecule with significant potential for clinical applications, we took advantage of phage-display technology to isolate a fully human single chain Fragment variable (scFv), named 4LB5, which binds with high affinity to the RNA binding domain (RBD) of NCL. In our previous study we demonstrated that 4LB5 binds NCL on the surface of aggressive breast cancer cells and inhibits their proliferation both in vitro and in vivo, representing the prototype of a new class of immune-based anti-NCL compounds.
Since NCL expression has been previously reported on the cell surface of skin cancer cell lines and up-regulation of NCL-dependent microRNAs was described in human melanomas, the objective of this project was the assessment of 4LB5 as a potential tool for melanoma therapy.

To this aim, the recombinant scFv was expressed as His6-fusion protein in E.Coli and purified by affinity chromatography, as previously described. By using Enzyme-Linked Immunosorbent Assays (ELISA), we demonstrated a significant binding of 4LB5 to the cell surface of different melanoma cell lines of both human and mouse origins. Notably, inhibition of NCL expression by siRNA transfection reduced the binding of 4LB5 to the cell surface of these cell lines, further supporting its specificity against NCL. Then, we assessed the potential effects of 4LB5 treatment on cell proliferation. Colony formation assays demonstrated that 4LB5 significantly affected cell proliferation of both human and mouse melanoma cell lines.

Our results, in agreement with previously reported data, further support the potential activity of 4LB5 as a tool for cancer therapy, paving the way for additional investigations aimed to fully elucidate the molecular mechanisms affected by this scFv and resulting in its anti-neoplastic therapy in human melanomas. Furthermore, this study supports the idea that anti-NCL immunoagents might represent a class of new anti-cancer compounds with a strong clinical relevance for a wide range of human tumors.

C172  Concomitant disruption of WEE1 and AurKA synergistically enhances anti-cancer effects in p53 mutated head and neck squamous cell carcinoma. Jong Woo Lee, Janaki Parameswaran, Ja Seok Koo, Barbara Burtness. Yale Cancer Center, New Haven, CT.

Head and neck cancer is a devastating disease which annually affects over half a million patients worldwide. Squamous cell carcinoma of the head and neck (SCCHN), which accounts for approximately 90% of all head and neck cancers, often harbors a disruptive mutation of the TP53 gene. Cells harboring TP53 mutations evade cisplatin-induced cellular senescence with cell cycle arrest in the G2 phase, allowing for DNA repair. Wee1 and Aurora Kinase A (AurKA) are crucial regulators of the mitotic cell cycle including G2/M phases and are significantly activated in SCCHN harboring TP53 mutations. Therefore, we hypothesized that a combination therapy aimed at these synthetic lethal targets, Wee1 and AurKA, would enhance anti-cancer effects and cisplatin sensitivity due to abrogation of G2 arrest concomitant with defects in tumor cell mitotic machinery, which is indeed caused by cisplatin. We tested this hypothesis using assays of cell viability, colony formation, anchorage independent cell growth, and finally a three-dimensional oncosphere formation assay in vitro. The combination index (CI) was calculated according to the Chou-Talalay method (CI<0.8, synergistic; 0.8<CI<1.2, additive; CI>1.2, antagonistic). The cell viability assay indicated that the combination of the Wee1 inhibitor MK-1775 with the AurKA inhibitor MLN8237 exhibited striking synergistic inhibitory effects on the cell growth of SCCHN cell lines including FaDu (TP53 mutation, CI=0.397004), Detroit562 (TP53 mutation, CI=0.202677) and UNC-7 (TP53 WT, CI=0.587106). Consistent with the cell viability data we obtained, we also confirmed this synergistic effect under anchorage dependent and independent cell growth conditions in FaDu and UNC-7 cell lines. Interestingly, this combination dramatically reduced the numbers of a stem cell-like oncosphere formation, showing a more effective combination in comparison to MK-1775 with cisplatin (inhibitory effect (% Vehicle): MK-1775+MLN8238 vs MK-1775+Cisplatin; FaDu= 74% vs 69%; UNC-7= 43% vs 36%). Moreover, all spheroids exposed to the combination of MK-1775 with MLN8237 did not survive following further incubation under normal cell culture conditions. Induction of apoptosis, as shown gradually increased PARP cleavage at Asp214-Gly215 in FaDu and UNC-7 cells, was demonstrated. Furthermore, this synergistic synthetic lethal effect was not seen in normal human tracheobronchial epithelial (NHTBE) cells in a three-dimensional organotypic (air-liquid interface culture condition) culture system, predicting low
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toxicity. These results suggest that SCCHN cells, including high-risk TP53 mutations, are sensitive to the combination of MK-1775 and MLN8237 in vitro, thereby supporting the preclinical and further clinical evaluation of MK-1775 in combination with MLN8237 for the treatment of patients with TP53 inactivated HNSCC.

C173 Anti-PD-L1 antibody-based IL-15 immunocytokine has enhanced antitumor immunity. Yan Wu¹, Zhaojing Zhong¹, Stella Martomo¹, Dan Lu¹, Zhanna Polonskaya¹, Xenia Luna¹, Haifan Zhang¹, Zhikai Zhang¹, Zhun Wang², Leo Liu², Jeegar Patel¹, James Tonra¹, Henry Li², Larry Witte¹, Sam Waksal¹, Zhenping Zhu¹. ¹Kadmon Corporation, NEW YORK, NY; ²Crown Bioscience, Inc., Santa Clara, CA.

Immune checkpoint antagonists to PD-1/PD-L1 and immunostimulating cytokines such as IL-15 have shown success to some extent in certain clinical settings across multiple cancer types. However, the full potential of the checkpoint inhibitor is limited due to impaired overall antitumor immunity, and cytokine as single agent has insufficient half-life and systemic toxicities due to the lack of target specificity. To overcome these challenging hurdles, we developed a bifunctional fusion protein, KD-033, composed of an antibody specific for PD-L1 and complex of IL-15Rα sushi domain/IL-15 as a novel immunotherapeutic agent for achieving better antitumor efficacy. Previously, we presented the generation and characteristics of a prototype of bifunctional fusion protein and its potential of in vivo antitumor activity. Here, we report a genetically modified fusion protein that has enhanced immunological activity and capability to achieve stronger antitumor efficacy in tumor models in comparison with either single agent. Our data indicate that the improved bifunctional fusion protein has favorable thermal stability and can be efficiently expressed in mammalian cells. The bifunctional fusion protein has higher affinity to PD-L1, silenced binding activity to Fc receptors and better ability to increase the secretion of Th1 cytokine, i.e. gamma IFN and the cytotoxicity of CD8 T-cells and NK cells to tumor cells as assessed in immunological assays. In preclinical study, KD033 had stronger anti-tumor efficacy in controlling primary tumor growth and prolonging the survival of tumor bearing mice in a number of mouse tumor models including those aggressive tumor models. Furthermore, the PD-L1 targeted bifunctional protein had significantly less cytokine-related toxicity when compared to non-targeted full IgG/IL-15Rα sushi domain/IL-15 fusion protein in vivo. These results demonstrate that KD033 has the capacity of targeting IL-15-stimulated innate and adaptive immune effectors into local tumor sites, thereby effectively controlling tumor progression while having minimized potential adverse effect in vivo. The preclinical studies of the novel immunotherapeutics warrant further investigation towards the clinical development of the bifunctional immunotherapeutic agent for cancer treatment.


Background: RAF and EGFR inhibitor combinations have shown promising clinical activity in patients with BRAF V600E colorectal cancer, but resistance invariably develops.

Methods: To define mechanisms of resistance to RAF/EGFR inhibition, we analyzed pre-treatment and disease progression samples from patients with BRAF mutant colorectal cancer using our custom next-generation sequencing platform, MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets). Cell line and patient derived xenograft models were generated to investigate the effects of the genetic alterations identified at disease progression.
Results: We identified alterations predicted to result in RAF dimerization and re-activation of ERK signaling in seven of eight post-progression tumors. These alterations included an intragenic deletion in BRAF encompassing the RAS binding domain, KRAS and NRAS mutations, and KRAS, NRAS, and BRAF amplifications. Notably, these alterations were rapidly enriched in the presence of drug treatment but selected against in the absence of drug exposure. A novel RAF inhibitor, that equipotently inhibits RAF mutant monomers and dimers, effectively suppressed ERK signaling in the RAF/EGFR resistant colorectal tumors at drug doses that did not inhibit ERK signaling in RAS/RAF wild-type cells. Treatment with this novel RAF inhibitor was able to overcome resistance in cell line and patient derived xenograft models.

Conclusions: Our data suggest that alterations that promote RAF dimers, which are resistant to first generation RAF inhibitors, are a unifying mechanism of RAF/EGFR inhibitor resistance in patients with BRAF mutant colorectal cancer. These results support the testing of RAF dimer inhibitors in combination with EGFR inhibitors in patients with BRAF V600E colorectal cancer.

Therapeutic Agents: Other


Tumor cells have an increased dependence on FASN-synthesized palmitate compared to non-tumor cells, which obtain many of their required lipids from the extracellular milieu. Palmitate and palmitate-derived lipids comprise diverse cellular components and function in processes required for tumor cell proliferation and survival. Previously we showed that FASN inhibition results in tumor cell apoptosis in vitro and xenograft tumor growth inhibition in vivo. Our studies demonstrated that diverse tumor types exhibit sensitivity to FASN inhibition and characterized mechanisms of action that associate with the antitumor activity of highly selective small molecule FASN inhibitors. In vitro studies with diverse tumor cell types elucidated a mechanism of action that includes plasma membrane remodeling, signal transduction pathway inhibition, and gene expression reprogramming.

TVB-2640 and TVB-3166 belong to a series of orally available, reversible, potent, and selective FASN inhibitors discovered and developed by 3-V Biosciences. Tumor xenograft studies were conducted in rats and mice to examine the in vitro and in vivo relationship of tumor cell sensitivity to FASN inhibition. Pharmacodynamic analyses of tumor and serum samples from these studies characterized the mechanism of action and biomarkers of sensitivity to in vivo FASN inhibition.

Once daily oral dosing of TVB-2640 or TVB-3166 caused inhibition of xenograft tumor growth for varied tumor models that included COLO-205 and HCT-116 colon adenocarcinoma cell lines. In vivo sensitivity to FASN inhibition was in agreement with in vitro data. Analysis of lipid, metabolite, protein, and RNA expression in tumor, blood or serum samples showed drug-induced modulation that was consistent with independent in vitro or in vivo studies. Lipid and metabolite changes included decreased palmitate and palmitate-associated lipids as well as increased expression of acylcarnitine species. Decreased expression of pAkt (S473), β-catenin, pβ-catenin (S675), and Myc proteins were found to associate quantitatively with xenograft tumor growth inhibition. Additionally, mRNA expression was modulated in a manner that revealed coordinated changes in the mRNAs from fatty acid, metabolism, cell survival, and cell growth-associated pathways. Expression changes in lipids, metabolites, proteins, and RNA species are leading to the development of a biomarker panel that describes FASN inhibitor target engagement and tumor sensitivity in
both in vitro and in vivo studies. These mechanism-based marker panels will be evaluated in current and upcoming clinical studies of TVB-2640.


Background: NEDD8 activating enzyme (NAE) is an essential E1 enzyme in the NEDD8 conjugation (neddylation) pathway, which controls cancer cell growth and survival through activation of cullin-RING ligase complexes (CRLs). Therefore, NAE is considered to be a promising target for cancer therapy. Here we describe the profile of a novel, highly potent, and selective NAE inhibitor, TAS4464, which inactivates CRLs in tumors and has strong antitumor efficacy in a preclinical model.

Materials and Methods: The selectivity of TAS4464 to E1 enzymes were assessed by various ubiquitin-like protein conjugation assays. The effects of TAS4464 on NEDD8 conjugation and the amounts of CRL substrates were evaluated by Western blot analysis. Cell viability was measured with a Cell Titer-Glo assay. The antitumor activity of TAS4464 was evaluated in an acute lymphoblastic leukemia (CCRF-CEM) xenograft model.

Results: We demonstrated that TAS4464 was a mechanism-based NAE inhibitor. TAS4464 formed an NEDD8-TAS4464 adduct that could achieve nanomolar inhibition of NAE. TAS4464 selectively inhibited NAE enzyme activity relative to the other E1s, ubiquitin activating enzyme (UAE) and SUMO activating enzyme (SAE). TAS4464 treatment inhibited cullin neddylation and induced accumulation of key CRL substrate proteins such as CDT1, p27, and phosphorylated IκB in human cancer cell lines. Enzyme inhibitory and cellular NAE inhibitory activities of TAS4464 were higher than those of a known NAE inhibitor, MLN4924. Cytotoxicity profiling of TAS4464 revealed widespread antiproliferative activity against a panel of cancer cell lines. Almost all cell lines derived from hematological malignancies were highly sensitive to TAS4464. Whereas carbonic anhydrase inhibition by MLN4924 resulted in accumulation in red blood cells, the blood-to-plasma ratio of TAS4464 was close to equivalent because of higher selectivity. In addition, TAS4464 did not inhibit major cytochrome p450 enzymes and showed good metabolic stability in isolated hepatocytes in vitro. The pharmacodynamic action of TAS4464 in tumors assessed by measuring the amount of cullin-NEDD8 conjugation in CCRF-CEM xenografts revealed strong cullin neddylation inhibitory activity of TAS4464; the inhibitory activity was sustained longer than that of MLN4924. In the CCRF-CEM xenograft model, weekly administration of TAS4464 at a dose of 100 mg/kg was more efficacious than twice-weekly administration of MLN4924 at a dose of 120 mg/kg: TAS4464 led to complete tumor regression without marked weight loss.

Conclusion: TAS4464 is the most potent and selective NAE inhibitor reported to date, with good pharmaceutical properties and superior efficacy. Hematological cancer cell lines are highly sensitive to TAS4464. Based on its promising preclinical activity, a phase 1 clinical trial of TAS4464 is being planned.

C177 TAS4464, a novel and highly potent inhibitor of NEDD8 activating enzyme, overcomes insensitivity to BTK, PI3Kδ, and IRAK4 inhibitors in activated B-cell like diffuse large B-cell lymphoma via inactivation of the NF-κB pathway. Hiromi Muraoka, Chihoko Yoshimura, Shingo Tsuji, Akihiro
Hashimoto, Takashi Mizutani, Shuichi Ohkubo, Kenichi Matsuo, Teruhiro Utsugi, Yoshikazu Iwasawa. Taiho Pharmaceutical Co., Ltd., Tsukuba, Japan.

Background: NEDD8 activating enzyme (NAE) regulates NEDD8 conjugation in neddylation pathway, a part of the ubiquitin-proteasome system, and controls cell cycle progression, DNA replication, and signal transduction via activation of cullin-RING ligases (CRLs) and subsequent degradation of their substrates. Through one such pathway, the constitutively active nuclear factor αB (NF-κB) triggers the progression of some types of hematological cancer. Activated B-cell like diffuse large B-cell lymphoma (ABC-DLBCL) remains the least curable form of DLBCL despite recent therapeutic advances. Because either phosphatidylinositol-3 kinase (PI3K)/AKT signaling or NF-κB signaling constitutively activated by B cell receptor signaling and MYD88 are reported to promote cancer cell growth and survival in ABC-DLBCL, the key kinases in these pathways [PI3Kδ, Bruton’s tyrosine kinase (BTK), and Interleukin-1 receptor-associated kinases (IRAK)] are considered to be potential targets for ABC-DLBCL therapy. We previously reported an NAE inhibitor, TAS4464, that could inactivate NF-κB by accumulating phosphorylated IκBα (p-IκBα). Here, we report on the possible application of TAS4464 to treat ABC-DLBCL that is insensitive to kinase inhibitors.

Materials and Methods: Human ABC-DLBCL cell lines, OCI-LY10, SU-DHL-2, and TMD8 were used to evaluate the biological activity of TAS4464. Intracellular ATP levels were measured to assess in vitro cell growth. The effects of TAS4464 on NEDD8 conjugation and levels of CRLs substrates were evaluated by Western blot analysis. The levels of NF-κB-targeted gene transcripts were assessed by qRT-PCR. The antitumor activities of TAS4464 were evaluated in both subcutaneous and systemic xenograft model of TMD8.

Results: TAS4464 led to growth arrest and death in ABC-DLBCL cell lines in the nanomolar range through inhibition of cullin neddylation. Similar effects were also observed in a cell line insensitive to the kinase inhibitors that inhibit divergent activator of NF-κB. qRT-PCR analysis revealed TAS4464 inhibited the NF-κB pathway in all ABC-DLBCL cell lines tested through accumulation of p-IκBα, which directly inhibit NF-κB activity. Intravenous administration of TAS4464 showed strong antitumor activity in a TMD8 xenograft model, with inhibited cullin neddylation and activation of caspases 3 and 8 in the tumors. Furthermore, in a TMD8 systemic model, intravenous administration of TAS4464 prolonged mice survival more than two-fold.

Conclusion: TAS4464 leads to cell growth through inhibition of NF-κB pathway regardless of the activation signaling pathway of NF-κB pathway. Though NF-κB activation signaling is complicated, the downstream inhibition of NF-κB pathway by TAS4464 indicated the potential for treatment of ABC-DLBCL in which NF-κB was dominant for their survival. In addition, TAS4464 demonstrates marked antitumor activity and survival benefit in a human ABC-DLBCL model. Therefore, TAS4464 may be a valuable addition to current options for chemotherapy in ABC-DLBCL.
Apoptotic proteins reportedly correlates with poor prognosis in AML, the modulation of apoptosis-related proteins emerges as a promising treatment strategy.

Apoptosis occurs mainly via two signaling pathways. In the mitochondria-mediated intrinsic apoptotic pathway, the release of cytochrome c triggers caspase-9 activation, which subsequently activates effector caspases (e.g., caspase-3). In the death receptor-mediated extrinsic apoptotic pathway, the binding of death receptor ligands to their receptors activates caspase-8 and, in turn, effector caspases, as in the intrinsic pathway. NEDD8 activating enzyme (NAE) inhibitors, which act on the NEDD8 conjugation pathway, induce quantitative changes in the substrates of E3 cullin-RING ligases (CRLs) and cause apoptosis in AML.

Here, we describe the mechanism by which our highly potent and selective NAE inhibitor TAS4464 promotes apoptosis in AML.

Materials and Methods: Intracellular ATP levels were measured to assess in vitro cell growth. The effects of TAS4464 on NEDD8 conjugation, the protein levels of CRL substrates, and the activation of various caspases were evaluated by means of Western blotting. The transcription levels of the Noxa and c-FLIP genes were assessed by using qRT-PCR. The antitumor activity and in vivo pharmacodynamic activity of TAS4464 were evaluated in a THP-1 xenograft model.

RESULTS: TAS4464 led to cell growth arrest and cell death in AML cell lines with various genetic backgrounds, including the MLL-AF9 fusion gene and FLT3-ITD mutation. TAS4464 activated both caspase-9 (intrinsic apoptotic pathway) and caspase-8 (extrinsic apoptotic pathway); combined treatment with inhibitors of these caspases markedly diminished the TAS4464-associated induction of apoptosis.

By analyzing apoptosis-related factors in each apoptotic pathway, we found that TAS4464 increased the pro-apoptotic factor Noxa protein (intrinsic apoptotic pathway) and decreased the anti-apoptotic factor c-FLIP protein (extrinsic apoptotic pathway) with changes evident at the mRNA transcriptional level. In addition, treatment with TAS4464 led to accumulation of the CRL substrate c-Myc, and c-Myc knockdown by siRNA ablated the TAS4464-induced decrease in cFLIP protein.

Intravenous administration of TAS4464 on day 1, 3 and 5 for 3 weeks resulted in tumor growth inhibition (TGI) of 100% including complete response in human THP-1 xenograft model, compared with twice-weekly administration of cytarabine that resulted in a TGI of only 6%. Pharmacodynamics analysis revealed that TAS4464 inhibited cullin neddylation and induced activation of caspases in THP-1 xenografted tumor.

CONCLUSION: TAS4464 exerts a strong apoptosis-inducing effect in AML cell lines via both intrinsic and extrinsic apoptotic pathway by modulating apoptosis-related proteins. In addition, TAS4464 demonstrates marked antitumor activity in the THP-1 xenograft model. Given its potent preclinical activities, TAS4464 is a promising agent for treating AML.
apoptosis by down-regulation of STAT3 activated genes. In this study we aimed to characterize a prodrug of GL, GPA512, with improved drug-like properties and to demonstrate its effect on tumor growth in a xenograft model of prostate cancer following oral administration.

Methods: Stability studies of prodrugs based on GL were performed in 0.1 M PBS buffer (pH 7.4) and in plasma at 37°C. In vitro efficacy of prodrugs was studied by WST-1 proliferation assay in DU145 prostate cancer cells expressing active STAT3. The systemic exposure of GL in mice was studied following a single oral dose of GPA512 or GL (both 10 mg/kg). The plasma concentrations of GL were determined by LC-MS/MS. For the xenograft study NMRI-nude male mice were inoculated subcutaneously with DU145 cells and once tumors were established the mice were divided in two groups with ten mice in each. Mice were treated orally with 40 mg/kg GPA512 daily five times/week for four weeks. Tumor growth was measured by caliper and at the end of the study tumors were harvested for subsequent analyses using immunohistochemistry and mRNA expression analysis.

Results: In vitro studies showed that the prodrug GPA512 is rapidly converted to GL in plasma and that GPA512 is stable in buffer solution and has similar inhibitory effects on proliferation as GL on DU145 cells. The pharmacokinetics of GPA512 following a single oral dose indicated that the compound was rapidly absorbed and converted to GL with a tmax of 15 min. Oral administration of GPA512 in mice increased the plasma exposure (AUC) of the active parent compound 20-fold compared to when GL was dosed orally. GPA512 treated mice bearing subcutaneous DU145 tumors had significantly smaller tumors compared to mice treated with vehicle. No adverse effects or weight loss were observed. Analysis of tumors showed decreased cell proliferation and increased amount of apoptotic cells in GPA512 treated mice compared to control. The mRNA expression of STAT3 regulated anti-apoptotic gene MCI-1 was significantly reduced by GPA512 treatment.

Conclusions: The drug-like properties and safety profile of the prodrug GPA512 and galiellalactone's direct inhibition of STAT3, warrant further studies of GPA512 as a drug candidate for treatment of patients with CRPC.

C181 RRx-001 combined with anti-PD-L1 antibody increases the complete response rate in a preclinical myeloma model. Susan J. Knox1, Shoucheng Ning1, Donna Peehl1, Bryan Oronsky2, Jan Scicinski2. 1Stanford University, Stanford, CA; 2EpicentRx, Inc., Mountain View, CA.

Background: RRx-001, a novel oxidizing and epigenetic agent, has shown promise as a single agent in a Phase I trial and is currently in Phase II trials, both alone and in combination with immunotherapy (Nivolumab), chemotherapy and radiation therapy.

Objective: The primary objective of the experiments described here was to test the hypothesis that RRx-001 combined with the checkpoint inhibitor, anti-PD-L1 would increase the complete response (CR) rate in a preclinical model of myeloma.

Methods and Results: BALB/c mice with well established J558L myeloma tumors (100-150 mm3 in volume) were treated with anti-PD-L1 antibody alone (10 mg/kg, i.p., twice a week for two weeks), RRx-001 alone (10 mg/kg, i.p., two doses on Day 0 and day 4) or the combination of anti-PD-L1 + RRx-001. Tumors were measured every other day and tumor response as well as the tumor volume quadrupling time (4X TGT) were calculated. In an initial pilot experiment with 4 mice/group, the combined therapy group had the best response compared to either treatment alone or the untreated control group. In a larger duplicate experiment with 8-10 mice/group, the CR rate was 0%, 37.5%, 22.2% and 75% for the untreated control, anti-
PD-L1, RRx-001, and combined group, respectively. In terms of a comparison of the 4X TGT, RRX-001 + anti-PD-L1 was more efficacious than the untreated control (P < 0.01), RRx-001 alone (P = 0.01) and anti-PD-L1 alone (P = 0.1).

Conclusions: These results demonstrate that inhibition of the PD-1/PD-L1 axis in combination with RRx-001 can significantly increase the CR rate in a preclinical model of myeloma. Experiments are ongoing to optimize this treatment regimen, study the effects of the combined treatment on the tumor immune microenvironment, and study the efficacy of this combined therapy in other tumor types. Results from this ongoing work will be presented, and have near term translational potential.

C182  Novel derivative of doxorubicin, AD198, for treatment of bladder transitional cell carcinomas in vitro. Dmitriy Smolensky, Kusum Rathore, Maria Cekanova, Maria Cekanova. University of Tennessee, Knoxville, TN.

Human bladder cancer is one of the most expensive cancers to treat due to its high rate of reoccurrence. Development and characterization of animal models for human cancers is important for the improvement of diagnosis and therapy. The bladder transitional cell carcinoma (TCC) of domestic animals resembles human bladder TCC in many aspects, therefore cell lines derived from bladder TCC of dogs are valuable model for studying human bladder TCC. A chemotherapeutic agent, Doxorubicin (Dox), is often used to treat advanced bladder cancer. Despite the success of Dox based therapies, drug resistance and cardio-toxicity are limiting factors in treating any cancer with Dox. N-benzyladriamycin-14-valerate (AD198), a novel derivative of Dox has no measurable cardio-toxic effects in the rodent model of lymphoma. In this study, we focused in comparing the efficacy and mechanisms of Dox and AD198 in human and primary canine bladder transitional cell carcinoma (TCC) cells in vitro. Using human T24 and UMUC-3 TCC and three canine primary bladder K9TCC-Dakota, K9TCC-Lillie, and K9TCC-Molly cell lines, we evaluated the Dox and AD198 efficacy on cell proliferation by MTS assay. Caspase 3/7 assay, reactive oxygen species (ROS) production, and western blot analysis were used to study the mechanisms of Dox- and AD198-induced apoptosis. AD198 was more effective than Dox in inhibition of cell proliferation in tested TCC cells in dose-dependent manner. ROS production was increased when compared to control by both Dox and AD198 leading to apoptosis, which was confirmed by caspase 3/7 activity and cleavage of poly ADP ribose polymerase (PARP) in tested TCC cells. AD198 increased ROS production significantly more than Dox in all tested TCC cells. Both Dox and AD198 increased phosphorylation of pro-apoptotic p38 MAPK, while at the same time increased the activity of anti-apoptotic phosphatidylinositol 3-kinase (PI3K/AKT/mTOR) signal transduction pathway in tested TCC cells. When a PI3K inhibitor, LY294002, was used in combination with either Dox or AD198, cell proliferation was inhibited more effectively than when drugs used alone. In addition, cleaved PAPR was increased when TCC cells were co-treated with LY294002 and in combination with Dox or AD198 as compared to either drug alone. Our data suggest that AD198 as novel derivative of Dox may be a valuable treatment option for bladder TCC cancers. Dox- and AD198-induced AKT phosphorylation that is an indicator of pro-survival and drug-resistance mechanisms of chemotherapies in tested bladder TCC cancer. Combined therapy of Dox or AD198 with inhibitors of PI3K/AKT1 pathway might lead to more effective treatment outcome for human and veterinary patients diagnosed with bladder TCC cancer. Evaluation of new therapeutic and imaging drugs that have shown promise in vitro and in vivo in the rodent model are important; however, pet animals like, dogs and cats with naturally-occurring tumors provide an important step for successful translation from rodents to human clinical application.

Glycolysis inhibition is an active area of investigation in cancer. However, few compounds have progressed beyond the cell culture stage. We have recently demonstrated that genomic passenger deletion of the glycolytic enzyme Enolase 1 (ENO1) leaves gliomas harboring such deletions with less than 10% of normal enzymatic activity, rendering them exquisitely sensitive to enolase inhibitors. However, the tool compound that we employed for these in vitro studies, Phosphonoacetohydroxamate (PHAH), has very poor pharmacological properties and was ineffective in vivo. We performed a SAR studies to increase inhibitor specificity towards ENO2 as well as pro-druging to increase cell permeability. The lead compound generated by these efforts, termed POMHEX, is selectively active against ENO1-deleted glioma cells in culture at ~35nM (versus 1µM for PPHA). Using an orthotopic intracranial xenografted model where tumor growth and response to therapy are monitored by MRI, we show that POMHEX is capable of eradicating intracranial ENO1-deleted tumors, with mice remaining recurrence-free even after treatment discontinuation. Taken together, these results reinforce that glycolysis is a viable target and provide in vivo proof-of-principal for the concept of using passenger deletions as targetable vulnerabilities in cancer therapy.

C184  BIND-510 improves the pharmacokinetics, tolerability, tumor accumulation and tumor growth inhibition in preclinical models of cancer compared to vincristine sulfate. Louise Cadzow, Kristen Arnold, Daniel Thrasher, James Nolan, Allen Horhota, Eric Lewis-Clark, James Wright, Susan Low. BIND Therapeutics, Cambridge, MA.

Background: BIND-510 is a novel, prostate-specific membrane antigen (PSMA) targeted Accurin polymeric nanoparticle encapsulating vincristine (VCR). PSMA is expressed on prostate cancer cells and on vasculature of many solid tumor types. VCR is an anti-mitotic agent most commonly used in the treatment of hematological cancers. Accurins have shown promise for targeting oncology agents preferentially to tumors, while limiting systemic exposure. To determine the potential benefit of encapsulation of VCR, BIND-510 was evaluated in pharmacokinetic (PK), tolerability, tumor accumulation and anti-tumor activity studies compared to VCR. The advantage of active targeting to enhance anti-tumor activity of BIND-510 was also explored in a PSMA expressing xenograft model. Immunohistochemistry was also performed to evaluate PSMA expression in hematological cancers.

Methods: VCR was encapsulated into nanoparticles using a nanoemulsion process to produce BIND-510. Single intravenous doses of BIND-510 were administered to rats for evaluation of PK. Tumor accumulation, tolerability and anti-tumor activity studies were performed in athymic mice implanted with breast cancer (MX-1), non-small cell lung cancer (NCI-H460), nasopharyngeal carcinoma (KB), PSMA-expressing prostate cancer (C4-2) and Burkitt’s lymphoma (Ramos) xenografts. PSMA immunohistochemistry was also performed in lymphoma and bone marrow tumor microarrays (TMA) to evaluate PSMA expression in hematological cancers.

Results: BIND-510 has a markedly different PK profile compared to VCR with increased exposure (300-fold) and decreased volume of distribution (500-fold) and clearance (300-fold). BIND-510 accumulated in KB tumors at an 8-fold higher concentration than VCR, and was shown to be more tolerable than VCR. BIND-510 administered to mice with KB xenografts as a single dose at the maximum tolerated dose (MTD) for VCR (1.5 mg/kg) resulted in a longer tumor growth delay compared to VCR. Due to improved tolerability, BIND-
510 could be administered at a dose of 3 mg/kg, resulting in complete responses in some mice, and even longer tumor growth delay than VCR or BIND-510 dosed at 1.5 mg/kg. Similar tolerability and anti-tumor activity data were obtained in MX-1 and Ramos xenografts. PSMA targeting of BIND-510 in C4-2 tumor xenografts resulted in a tumor growth inhibition (TGI) of (79%) compared to non-targeted VCR nanoparticles which caused a 47% TGI at the same VCR dose level. NCI-H460 tumor xenografts are insensitive to VCR at MTD, but are sensitive to BIND-510 due to improved tolerability at higher dose levels. PSMA immunostaining was detected in the neovasculature of 27.5% diffuse large B cell lymphoma tumors and 35% of multiple lymphoma tumors analyzed.

Conclusions: BIND-510 exhibits differentiated PK, tumor accumulation, tolerability and anti-tumor activity compared to VCR. In combination with the demonstration of PSMA expression in hematological cancers, our findings support the feasibility of developing BIND-510 as a targeted clinical therapy with the potential benefit of reduced toxicity and improved anti-tumor activity compared to currently available treatments in hematological as well as solid tumor settings.

C185 Targeting PARP and DNA as a novel unimolecular combination model for the enhancement of chemosensitivity. Zhor Senhai Mouhri, Elliot Goodfellow, Bertrand Jean-Claude. The Research Institute of The McGill University Health Centre, Montreal, QC, Canada.

The concept of synthetic lethality arises when the alteration of a single gene A or B alone does not affect cell survival but that of both genes lead to cell death. More importantly, if A is mutated, inhibition of the functions of the gene B product should also lead to cell death. This is achievable in some tumours, predominantly in breast and ovarian cancers, where the mutation of the DNA repair proteins BRCA1/2 depletes the DNA repair capacity of the cells. The compensatory DNA repair mechanism in the cells is supported by a DNA repair enzyme poly(ADP-ribose) polymerase (PARP), which when inhibited, leaves the cells with no compensatory DNA repair mechanisms. Hence DNA lesions accumulate in the cells and ultimately cause their death. The ability of PARP inhibitors to induce cell death in BRCA1/2 mutated tumours has been demonstrated in both preclinical models and in the clinic. While the PARP inhibitor olaparib met the requirements for recent FDA approval for the treatment of ovarian cancer, the overall survival obtained with many PARP inhibitors as single agents in patients with BRCA1,2 mutated tumours has been disappointing. Also, acquired resistance caused by the reactivation of wild type BRCA1/2 is an emerging drawback in BRCA1/2 related therapies. Our hypothesis is that molecules termed “combi-molecules” capable of not only inhibiting PARP but also inducing DNA damage, could significantly enhance cell-killing in BRCA1/2 deficient tumours and perhaps be indicated at the earlier stages of the diseases. Here, we designed and studied the potency and mechanism of action of our first prototype EG22. The results showed that: (a) it inflicted higher levels of DNA damage than temozolomide (a clinical DNA damaging agent) and >100-fold stronger growth inhibitory potency than the latter in our cell panel, regardless of the BRCA1/2 status of the cells, (b) it showed more than 5-fold stronger growth inhibitory potency than 4-ANI, a known PARP inhibitor, in BRCA1/2 deficient cells and more than 8-fold in wild type expressing cells (c) a PARP assay showed that it induced a dose-dependent inhibition of PARP with an IC50 value almost equal to that of 4-ANI and (d) in cell-based selectivity assay involving a pair of isogenic Chinese hamster cell line (engineered to express BRCA2 wild type), it induced 20-fold selectivity for the mutant form. The results in toto suggest that EG22 is a new molecular entity with a novel and dual mechanism of action. Furthermore, reactivation of BRCA1/2 being one of the mechanisms of resistance to DNA damage-based therapy of patients with mutant BRCA1/2, the combi-molecular approach has the potential to be developed as an alternative treatment, when the tumours heterogeneously express BRCA1/2.
Therapeutic Agents: Small Molecule Kinase Inhibitors

**C186** SNS-062 is a potent noncovalent BTK inhibitor with comparable activity against wild type BTK and BTK with an acquired resistance mutation. Minke E. Binnerts¹, Kevin L. Otipoby², Brian T. Hopkins², Tonika Bohnert², Stig Hansen¹, Gene Jamieson¹, Pamela A. Howland¹, Eric H. Bjerkholt¹, Deborah A. Thomas¹, Judith A. Fox¹, Adam R. Craig¹. ¹Sunesis Pharmaceuticals, Inc., South San Francisco, CA; ²Biogen, Cambridge, MA.

Background and purpose: BTK mediates B-cell receptor signaling and was validated as a target by the BTK inhibitor ibrutinib in several B-cell malignancies, including mantle cell lymphoma and chronic lymphocytic leukemia (CLL). However, patients may have or acquire resistance. Resistance mechanisms include mutation of the cysteine in the BTK active site that ibrutinib requires for covalent binding (C481). We identified and characterized SNS-062, a potent, noncovalent BTK inhibitor with activity towards BTK harboring resistance mutations that also inhibits ITK and may provide enhanced anti-tumor immune responses. SNS-062 shows restricted kinase selectivity and nonclinical pharmacology, pharmacokinetics (PK) and toxicology profiles distinct from ibrutinib and merits clinical investigation. Methods and Results: In in vitro kinase binding assays, SNS-062 bound to 9 kinases with Kd < 25 nM, including Tec kinase family members BTK (0.3 nM) and ITK (2.2 nM), but did not bind EGFR. Lack of EGFR inhibition by SNS-062 may offer safety advantages over ibrutinib, which has been associated with diarrhea and rash potentially related to its off-target effects on EGFR. Cellular inhibition of BTK was demonstrated by measuring inhibition of BTK auto-phosphorylation. SNS-062 inhibited pBTK in human whole blood with an average IC50 of 50 nM. A PK/ PD (pBTK) relationship was demonstrated in mice, with an in vivo IC50 for pBTK inhibition of 47 nM. Prolonged SNS-062-mediated in vivo inhibition of pBTK correlated with potent efficacy in a T cell-independent (type II) BTK dependent B cell mediated antibody response mouse model (ED50 11 mg/kg); efficacy was also observed in a type II collagen- induced arthritis (CIA) rat model (ED50 for summed knee and ankle histopathology 0.8 and 3.9 mg/kg). To assess the activity of SNS-062 and ibrutinib against BTK with acquired resistance mutations, inhibition of wild type (WT) and mutant C481S BTK was evaluated in direct kinase assays. SNS-062 inhibited WT and C481S BTK with similar IC50s (pBTK IC50s: WT BTK 2.9 nM, C481S BTK 4.4 nM) while ibrutinib potency was reduced 40-fold (pBTK IC50s: WT BTK 0.58 nM, C481S BTK IC50 25.7 nM). Similarly, ibrutinib showed a 100-fold loss of potency in C481S BTK expressing 293 cells (pBTK IC50s: WT BTK 0.016 \(\mu\)M, C481S BTK 1.7 \(\mu\)M) while SNS-062 activity was unaffected (pBTK IC50s: WT BTK 0.57 \(\mu\)M, C481S BTK 0.80 \(\mu\)M). SNS-062 had good oral bioavailability in rat and dog (%F ≥ 40%) and a terminal half- life of 5 to 6 hours. 28 day repeat-dose toxicology studies in rat and dog showed that SNS-062 was well tolerated with continuous drug levels and at exposures (AUC∞) much greater than those achieved for ibrutinib. These results suggest that SNS-062 plasma concentrations may be achieved that provide prolonged inhibition of both WT and mutant BTK. Conclusions: SNS-062 is a noncovalent BTK inhibitor that also inhibits ITK but not EGFR. SNS-062 activity against both WT and mutant C481S BTK was similar, while ibrutinib activity was decreased 40 to 100-fold. SNS-062 has a nonclinical PK/safety profile that will likely provide SNS-062 plasma concentrations for sustained inhibition of both WT and mutant BTK. These results support clinical development of SNS-062 for B cell malignancies, including CLL with acquired resistance to ibrutinib.
Cross-inhibition of Trk receptors by TAS-119, a novel Aurora A selective inhibitor, exhibits therapeutic potential in a Trk-driven cancer model. Hiroshi Sootome1, Akihiro Miura1, Kimihiro Ito1, Takamasa Suzuki1, Hiroshi Hirai2, Teruhiro Utsugi2, Kazuhiko Yonekura2. 1taiho Pharmaceutical Co., Ltd., Tsukuba, Japan; 2taiho Pharmaceutical Co., Ltd., Tokyo, Japan.

Background; Comprehensive analysis by next-generation sequencing has identified oncogenic gene rearrangement of Trk neurotrophin receptors TrkA, TrkB and TrkC (encoded by NTRK1, NTRK2, and NTRK3 gene, respectively) in multiple tumor types including lung adenocarcinoma, colorectal cancer, glioblastoma and acute myeloid leukemia. The creation of gene fusion through genomic rearrangement results in constitutive activation of the kinase domain of Trk. These recent findings encourage study of the therapeutic potential of Trk kinases as drug targets for the specific treatment of patients with oncogenic NTRK gene abnormalities. TAS-119 is a novel small molecular weight inhibitor of Aurora A kinase and currently investigated in phase 1 clinical trial as both an enhancer of microtubule stabilizing drugs and monotherapy (1-3). In addition, TAS-119 shows pan-Trk inhibition with potency similar to Aurora A inhibition. Here, we describe the in vitro and in vivo pharmacologic profile of TAS-119 as a pan-Trk inhibitor.

Methods; Kinase assays were performed with purified recombinant enzymes under optimized conditions using ATP concentration at or just below the respective Km. In cell proliferation studies, cell viability was determined at 72 hour by detecting cellular ATP concentration. Analysis of protein expression including phosphorylated proteins was conducted by Western blotting. In tumor xenograft models, cells were implanted subcutaneously in nude mice. TAS-119 was administered orally with twice daily or intermittent dosing (4 days on/3 days off schedule) for 14 days.

Results; TAS-119 inhibited TrkA activity with an IC50 of 1 nM in cell-free system, and inhibited TrkA autophosphorylation and cell proliferation of KM12C colorectal cancer cell line harbouring TPM3-TrkA fusions at sub-nM range. In the KM12C xenograft model, both continuous and intermittent treatment with TAS-119 at 60 mg/kg or 100 mg/kg b.i.d. for 14 days resulted in dose-dependent inhibition of tumor growth with good tolerability. Delays of tumor growth in xenograft model were well correlated with reduction of TrkA autophosphorylation and inhibition of downstream signal transduction like pPLCγ, pERK and pAKT within tumors.

Conclusions; TAS-119 is an orally bioavailable dual-specificity kinase inhibitor developed specifically for both Trk and Aurora A inhibition with favorable profiles in animals. These preclinical data suggest that TAS-119 has therapeutic potential for cancers with Trk rearrangements. Currently, phase 1 dose-escalation clinical trial is ongoing. Although TAS-119 is primarily being developed as an inhibitor for Aurora A kinase, it should also be tested clinically in patients with tumors showing Trk gene rearrangement.

1) 24th EORTC-NCI-AACR Symposium (2012), abstract #251 and #252
2) 25th EORTC-NCI-AACR Symposium (2013), abstract #A268 and #A269
3) 26th EORTC-NCI-AACR Symposium (2014), abstract #P005 and #P178
Background: RXDX-105 is a potent RET, BRAF and EGFR tyrosine kinase inhibitor (TKI) that exhibits high target affinity at low nanomolar concentrations. The RET proto-oncogene encodes a receptor tyrosine kinase for members of the glial cell line-derived neurotrophic factor (GDNF) family of extracellular signaling molecules. RET gain of function alterations are associated with the development of various types of human cancer, including non-small cell lung cancer (NSCLC). BRAF plays a key role in regulating the MEK/ERK signaling pathway, which affects cell division and differentiation. Acquired BRAF mutations can result in constitutive activation of this pathway, thereby fueling cancer growth. RXDX-105 is being developed as an oral therapy for patients with solid tumors that harbor RET or BRAF mutations or gene rearrangements.

Methods: Adult pts with advanced solid tumors were enrolled in a Ph 1 single agent dose escalation study with a standard 3 + 3 design to determine the recommended Ph 2 dose. Oral RXDX-105 was given as a fixed dose on a continuous daily schedule. Tumor response was assessed at baseline and every 8 wks (RECIST v1.1). Treatment-emergent adverse events (AEs) were recorded according to NCI CTC v4.03. Pharmacokinetic (PK) assessment was also a study objective.

Results: To date, 35 pts (17 M and 18 F) received RXDX-105 across 7 dose levels (20 to 275 mg QD). Median age was 60.5 years (range 27-81). The most frequent tumors (pts) were metastatic CRC (13), ovarian cancer (3), NSCLC (3), cholangiocarcinoma (3), and pancreatic cancer (3). Median number of cycles was 2 (range 1 to 23).

34 pts experienced AEs; 6 pts experienced treatment-related G3 AEs, including anemia, hypophosphatemia, fatigue, diarrhea, abdominal pain, rash and muscle weakness. 22 SAEs were reported from 9 pts, with none considered treatment-related. No treatment-related deaths occurred. The most common AEs were: fatigue (17 pts), vomiting (13 pts), abdominal pain (12 pts), nausea (11 pts), decreased appetite (10 pts), and rash (10 pts). At the time of this report, 3 pts have had DLTs; 1 pt at 200 mg with G2 hand and foot syndrome, 1 pt at 275 mg with G3 fatigue, and 1 pt at 275 mg with G3 asymptomatic hypophosphatemia. The protocol was amended to exclude hypophosphatemia as a DLT since it has been observed with approved TKIs and can be managed by supplementation.

The PK data to date have demonstrated that RXDX-105 is absorbed with a median Tmax between 2 and 6 hrs at steady state. RXDX-105 plasma concentrations declined slowly after reaching Cmax with an average terminal half-life of 28 to 42 hrs across dose levels. There was a loss of dose proportionality at doses above 150 mg, possibly due to pH-dependent drug dissolution in the stomach. The impact of the fed state on exposure is being explored.

11 pts have been on treatment for ≥ 12 weeks. No objective responses have been observed; however, 1 pt with BRAF V600E-positive papillary thyroid cancer, previously treated with RAI, EBRT and multiple surgical resections, has been on study with stable disease for almost 2 years (23 cycles). Additionally, 2 heavily pre-treated pts with squamous NSCLC achieved clinical benefit with SD for > 6 months; 1 patient continues.

Conclusions: To date, treatment with RXDX-105 demonstrates an acceptable safety profile. Improvements in bioavailability are being investigated. Dose escalation continues.
C189  Defective S phase cell cycle checkpoint: A potential culprit and target in melanoma. Zay Y. Oo, Sheena Daignault, James Chen, Brian Gabrielli. The University of Queensland Diamantina Institute, Translational Research Institute, Brisbane Queensland, Australia.

The S phase cell cycle checkpoint is critical for maintaining genomic integrity. This checkpoint is triggered in response to DNA damage and replication stresses to control cell cycle progression, halting DNA replication and allowing the time to repair to maintain genomic integrity. If this checkpoint is defective, cells lose their ability to ensure the fidelity of replication and repair any damage, resulting in replicative stress and genomic instability which will subsequently increase the risk of cancer. We have discovered that a large proportion of melanoma cell lines lose the ability to halt replication when challenged with high dose hydroxyurea-induced replicative stress, despite normal cell cycle checkpoint signalling. We found that failure in the crosstalk between ATR-CHK1 checkpoint signalling and the cell cycle mechanism in destabilization of CDC25A. Although CHK1-dependent phosphorylation of CDC25A which triggers its degradation was detected, the S phase checkpoint defective melanoma cell lines fail to destabilize CDC25A, maintaining normal activation of CDK2/Cyclin E. The melanoma cells with this S phase checkpoint defect are also hyper-sensitive to killing by CHK1 inhibitor. We have previously shown that melanoma with high levels of replicative stress are very sensitive to CHK1 inhibitors. I will report our studies investigating whether CHK1 inhibitor sensitivity is a direct outcome of the replicative stress arising from the S phase checkpoint defect, or whether the loss of the ATR-CHK1-CDC25A dependent S phase checkpoint signalling mechanism is directly responsible for the hyper-sensitivity to CHK1 inhibitors.


Cyclin-dependent kinase 7 (CDK7) is an important constituent of the cellular transcriptional machinery, where it phosphorylates the C-terminal domain (CTD) of RNAP polymerase II (RNAPII). Because many tumor types are critically dependent on transcription for maintenance of their oncogenic state, pharmacological modulation of CDK7 kinase activity is considered as an approach to treat cancer. Multiple series of CDK7 inhibitors were identified by iterative medicinal chemistry efforts and SAR based approach. Early compounds were optimized towards attaining good physicochemical properties, high potency, good selectivity and desirable pharmacokinetic profile to achieve anti-tumor activity. We have identified compounds from two distinct chemical series that are highly potent in inhibiting CDK7 in biochemical assays. These inhibitors demonstrate time-dependent inhibition of CDK7 indicating covalent nature of binding. The compounds showed potent anti-proliferative activity in cell lines derived from various tumor types and this was accompanied by CDK7 modulation in cells as monitored by pS5RNAPII levels. They have excellent drug-like characteristics including solubility, permeability, metabolic stability and good oral bioavailability. In a broad panel of kinases (332 kinase), selected compounds from both series showed good selectivity profile. Tolerability and efficacy studies are ongoing with selected early leads to test their impact on tumor growth inhibition in xenograft models. We have identified novel and selective CDK7 covalent inhibitors from two series with desirable drug-like properties, which are being evaluated for anti-tumor activity in xenograft models.

Interleukin-1 receptor associated kinases (IRAKs) are serine/threonine protein kinases belonging to tyrosine-like kinase (TLK) family. The IRAK family consists of IRAK1, IRAK2, IRAK3 and IRAK4 out of which only IRAK1 and IRAK4 exhibit kinase activity. IRAKs function as mediators of Toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) signaling pathways and play an important role in innate immune signaling. Recent studies have reported the occurrence of oncogenic mutations in MYD88 in 30% of activated B cell diffuse large B-cell lymphoma (ABC DLBCL) and 90% of Waldenstrom's macroglobulinemia (WM) leading to constitutive activation of the IRAK4 and NFkB pathway. Recent studies have also highlighted the association of dysregulated innate immune signaling with Myelodysplastic syndrome (MDS) and Acute Myeloid leukaemia (AML). TLRs and their associated signal transducers are frequently overexpressed and/or constitutively activated in MDS. Overexpression and activation of IRAK1 is observed in AML. Thus IRAKs are attractive therapeutic targets for treatment of tumors with altered innate immune signaling such as ABC DLBCL and AML.

We have designed, synthesized and tested small molecule inhibitors of IRAK4 based on hits originating from Aurigene’s compound library. We have identified a series of novel bicyclic heterocycles as potent inhibitors of IRAK-4 with moderate to very high selectivity (S35 score = 0.03) in a 329 kinase panel. Lead compounds were profiled in proliferation and mechanistic assays (p-IRAK1 and p-TAK1 inhibition) in appropriate ABC DLBCL/AML cell lines. Aurigene lead compounds demonstrate potent inhibition of cellular proliferation with a good correlation to inhibition of phosphorylation of signaling intermediates in mechanistic assays. Lead compounds exhibit excellent PK profile and good oral bioavailability in mice. Preliminary in-vitro toxicology studies indicate a clean safety profile. Selected compounds demonstrate excellent in-vivo efficacy in relevant tumor models with >90% tumor growth inhibition and good in-vivo PD modulation. In summary, a series of potent and selective IRAK4 inhibitors have been discovered and are being evaluated for treatment of cancers with dysregulated innate immune signaling.

C193  Combining ibrutinib with Chk1 inhibitors as a new therapeutic strategy for mantle cell lymphoma. Valentina Restelli1, Micaela Vagni1, Monica Lupi1, Francesco Bertoni2, Giovanna Damia1, Laura Carrassa1. 1IRCCS- Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy; 2Lymphoma and Genomics Research Program, IOR Institute of Oncology Research; Lymphoma Unit, IOSI Oncology Institute of Southern Switzerland, Bellinzona, Switzerland.

Mantle cell lymphoma (MCL) is an aggressive B-cell lymphoma characterized by an initial high response to first line treatment, followed by a continuous relapse, resulting in a discouraging long term outcome. Recently a novel potent inhibitor of BTK, ibrutinib, was found to induce impressive response in B-cell malignancies and has been approved for therapy of refractory mantle cell lymphoma. BTK (bruton's tyrosine kinase) is required for B cell receptor signaling (BCR) and is essential for B cell development, differentiation, signaling and survival. Despite its unprecedented clinical activity, acquired resistance to ibrutinib also develops, pushing the evaluation of new drug combinations. We have recently shown that Chk1 inhibitors (PF-00477736) not only are very effective as single agents in MCL but are strongly synergistic with Wee1 inhibitors. We tested ibrutinib in combination with PF-00477736 in different MCL cell lines (JVM2, Jeko-1, MAVER-1, GRANTA-519, RECI), finding that the combination is synergistic in all these cell lines, with Combination Index values, evaluated at the IC50 doses, ranging from 0.5 to 0.7. The combination was also
very active in a JEKO-1 cell line made resistant to PF-00477736. The synergistic effect of the combination was also observed using another Chk1 inhibitor (AZD-7762). Western Blot Analysis revealed that the combined treatment at concentrations of ibrutinib and PF-00477736 not toxic as single agents, neutralized the increase in phosphor-ERK observed after PF-00477736 treatment, and led to a substantial decrease in the active phosphorylated form of BTK in Y223. Cell cycle analysis and apoptosis are undergoing to try to dissect the cytotoxic mechanisms induced by the combination along with studies aiming at elucidating the molecular mechanisms at the basis of this combination. These data would suggest that ibrutinib in combination with Chk1 inhibitors could be a new and effective therapeutic strategy to test in clinical setting in MCL.


Herewith, we report development of small molecule inhibitors of MNK1 and MNK2 kinases and their cellular activity. MNK1 and 2 are MAP kinase-interacting kinases are activated by RAS and MAPK signaling pathways, and are involved in regulation of translation. Both kinases phosphorylate translation initiation factor eIF4e on a conserved serine 209. eIF4E can contribute to the oncogenic transformation both in vitro and in vivo and is highly expressed in diverse types of cancer. Interestingly, mice that lack both Mnk1 and Mnk2 do not have any apparent phenotype. Recently first dual MNK1/MNK2 inhibitors have entered clinical trials as a combinational therapy with docetaxel in NSCLC.

SEL201 is a series of small molecule inhibitors which inhibit activity of both MNK1 and MNK2 in a low nM range and high selectivity confirmed in kinome panels. Analysis of SEL201 cellular activity indicated potent inhibition of eIF4e Ser209 in vitro in cancer cells and in vivo after oral administration in xenograft tumors. Repressed phosphorylation of eIF4e resulted in impaired translation of several proteins involved in metastasis and activation of immune cells. High potency, selectivity and favorable ADME/PK profile indicates that SEL201 inhibitors would be useful tools in probing molecular consequences of eIF4e Ser209 inhibition in cancer cells. SEL201 in vitro and in vivo activities on viability and metastasis will be presented in cellular and in vivo models of solid tumors and hematological malignancies. SEL201 series is further developed as a cancer therapy with a good therapeutic window.

C195 A Wee1 inhibitor analog of AZD1775 demonstrates synergy with cisplatin with reduced single-agent toxicity in medulloblastoma. Christopher J. Matheson, Sujatha Venkataraman, Vladimir Amani, Peter Harris, Donald S. Backos, Nicholas K. Foreman, Rajeev Vibhakar, Philip Reigan. University of Colorado, Aurora, CO.

Medulloblastoma is the most common primary brain tumor in children. Current treatment for medulloblastoma includes surgical resection, radiation and cytotoxic chemotherapy. Although this approach has improved survival rates, the high doses of chemotherapy required to circumvent drug resistance mechanisms and result in clinical efficacy often give rise to lasting neurocognitive defects, stunted growth, deafness, and even secondary tumors. Therefore, synergistic drug combinations that maintain clinical efficacy, but allow dose reductions of cytotoxic agents limiting their adverse effects would be an attractive approach for patients with medulloblastoma. We identified Wee1 kinase as a new molecular target for medulloblastoma from an integrated genomic analysis using pathway analysis of gene expression and a
kinome-wide siRNA screen of medulloblastoma cells and tissue. Wee1 participates in the G2-M checkpoint to prevent mitosis in the presence of DNA damage and therefore may play a role in drug resistance to DNA alkylating agents, such as cisplatin. Our data indicate that Wee1 prevents DNA damage-induced cell death by cisplatin and that the known Wee1 inhibitor AZD1775 (previously MK1775) displays synergistic activity with cisplatin. However, AZD1775 is known to have nanomolar activity with at least 8 other kinases and there is limited structure-activity relationship (SAR) data for AZD1775 as it was identified as a Wee1 inhibitor from a high-throughput screen. Therefore, we developed a small series of AZD1775 derivatives to establish a SAR and further examine the effects of Wee1 inhibition in medulloblastoma. The compounds, that inhibited Wee1 activity in a TR-FRET assay in the same nanomolar range as AZD1775, had significantly reduced single-agent cytotoxicity and displayed synergistic activity with cisplatin at lower concentrations than AZD1775 in medulloblastoma cells. Recently, it has been reported that AZD1775 has limited ability to diffuse across the blood-brain barrier (BBB) and this would limit its effectiveness in brain tumors. We have performed cell permeability and BBB permeability assays using AZD1775 and our lead Wee1 inhibitor and our studies support that AZD1775 has poor BBB penetration. Our studies have now transitioned into in vivo systems to determine the tissue distribution and pharmacokinetics of our lead Wee1 inhibitor compared with AZD1775. In addition, our studies will determine the effect of our lead Wee1 inhibitor and AZD1775 on tumor growth as single agents and in combination with cisplatin in medulloblastoma in vivo models.

C196 Identification of first-in-class, highly potent FGFR kinase inhibitors that spare FGFR1. Shannon Winski1, Nisha Nanda2, Eric Brown1, Tony Tang1, Barbara Brandhuber1, Robyn Hamor1, Brian Tuch2, Kevin Ebata2, Jennifer Low2, Francis Sullivan1, Darin Smith1, Guy Vigers1, Megan Strough1, Rob Rieger1, James Blake1, David Moreno1, David Chantry1, S. Michael Rothenberg2, Steven Andrews1. 1Array BioPharma, Boulder, CO; 2LOXO Oncology, Stamford, CT.

Background: The fibroblast growth factor receptor (FGFR1-4) family of tyrosine kinases plays an important role in normal physiologic processes, including angiogenesis, wound healing and regulation of calcium and phosphate metabolism. In addition, dysregulation of FGFR signaling through genetic alterations or altered expression of individual receptors and their ligands has been frequently observed in human tumors. While tyrosine kinase inhibitors (TKIs) with anti-FGFR activity have produced clinical responses in patients whose tumors harbor FGFR alterations, currently available FGFR TKIs inhibit multiple other kinases, including multiple FGFRs. As a result, dose-limiting toxicities have been frequently observed in patients, including hyperphosphatemia which may arise from the inhibition of FGFR1 in the kidney. These toxicities may ultimately limit the efficacy of pan-FGFR inhibitors. However, the ability to develop inhibitors that spare individual FGFRs has been hampered by the high degree of structural similarity between FGFR1, FGFR2, and FGFR3. The development of these tools to distinguish the functional contributions of the individual FGF receptors not only advances the biological understanding of the individual receptors in the context of their expression but may also provide therapeutic agents that have an improved therapeutic index.

Methods: In vitro and in vivo evaluations including both enzyme and cell-based assays, pharmacokinetic (PK) studies, measurement of drug metabolism and non-clinical safety evaluation were conducted using standard methods. Tumor growth inhibition and pharmacodynamics (PD) measurements were carried out using subcutaneous xenografts of RT112/84 (which harbor an FGFR3-TACC fusion) bladder cancer cells in nude mice.

Results: We have developed compounds with nanomolar FGFR3 enzyme and cell potency, but relatively spare FGFR1 and have minimal activity against an enzyme panel of >200 diverse kinases. This series was further optimized to provide high oral exposure in rodent species. One representative compound was
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evaluated in a single dose mouse PK at doses of 10, 30 and 100 mg/kg and provided predicted pharmacokinetic coverage of the FGFR3 cell IC50 for >8, >12 and >24 hrs respectively, which correlated with phospho-FGFR3 reduction. The compound demonstrated greater than 40% and greater than 65% tumor regressions of RT112/84 subcutaneous xenografts at doses of 30 and 45 mg/kg/day x 14 day, respectively. These doses were well tolerated and minimal hyperphosphatemia was observed.

Conclusions: We have identified potent and selective FGFR inhibitors that spare FGFR1 and other related kinases, and possess high oral bioavailability and favorable PK properties in animals. This next-generation class of compounds will be able to examine selectively the biology of selective FGFR inhibition. The identification of potent and selective FGFR inhibitors with minimal activity against FGFR1 may improve the efficacy and tolerability compared to the currently available pan-FGFR inhibitors.

C197 Accurins improve the pharmacokinetics, pharmacodynamics, tolerability and anti-tumor activity of the AKT inhibitor MK-2206. Louise Cadzow1, Michael H. Lam2, Young Ho Song1, Maria Figueiredo1, Hong Wang1, David De Witt1, Vincenzo Pucci2, Jan-Rung Mo2, Eric Lewis-Clark2, Heidi Ferguson2, Marian Gindy1, Susan Low1, Steve Zale1, Jeff Hrkach1, Caroline McGregor2, Brian J. Long2. 1BIND Therapeutics, Cambridge, MA; 2Merck & Co., Inc, Boston, MA.

Background: BIND-2206 Accurins are novel polymeric nanoparticles encapsulating MK-2206, a Merck AKT inhibitor in phase 2 trials. MK-2206 targets the phosphatidylinositol 3-kinase (PI3K) pathway via AKT inhibition and has demonstrated therapeutic efficacy in the treatment of cancer albeit with dose limiting toxicities. Accurins have shown promise for targeting oncology agents preferentially to tumors, while limiting systemic exposure. To determine if encapsulation of MK-2206 improves therapeutic index, studies were performed to evaluate pharmacokinetics (PK), pharmacodynamics (PD), tolerability and anti-tumor activity compared to parent MK-2206.

Materials and Methods: PK of four Accurin formulations with varying in vitro drug release rates was evaluated. Based on these data, two lead Accurins (BIND-2206C and BIND-2206D) were selected for further characterization. Since AKT inhibitors are documented to induce hyperglycemia in mice, blood glucose levels were also evaluated after acute administration of BIND-2206 Accurins or parent MK-2206 as a measure of tolerability. Using a panel of human tumor xenografts (VCAp, SK-OV-3 and BT-474) anti-tumor activity of the Accurins was assessed in mice. When tumors were established mice were dosed orally with parent MK-2206 or intravenously with BIND-2206 Accurins two or three times per week on a three week cycle. Tumor volume was measured post treatment to determine anti-tumor activity. Following final drug administration, tumors were collected for PK and PD evaluation. Extent and duration of tumor target inhibition was evaluated by measuring pAKT and total AKT using a mesoscale discovery method and was correlated to tumor MK-2206 levels.

Results: Accurins BIND-2206C and BIND-2206D significantly enhanced the PK profile of parent MK-2206 by increasing Cmax (33 fold) and AUC (222 fold) and decreasing clearance (38 fold) in mice. In addition, both BIND-2206 Accurins improved tolerability as demonstrated by no hyperglycemic response compared to a 300% increase in blood glucose in nude mice treated with parent MK-2206. In the VCAp human prostate cancer tumor model, BIND-2206C inhibited tumor growth by 88% and BIND-2206D induced 16% tumor regression showing significant enhancement of anti-tumor efficacy compared to 55% tumor growth inhibition achieved by parent MK-2206. This correlated with high tumor exposure at 72 hours and prolonged tumor PD at 96 hours post dose for both Accurins compared to parent MK-2206. A similar increase in tumor exposure and prolonged PD response resulted in enhanced anti-tumor efficacy for BIND-2206 Accurins in
the SK-OV-3 human ovarian cancer tumor model. In the BT-474 HER2 over-expressing human breast cancer tumor model, BIND-2206 Accurins did not improve efficacy compared to parent drug, although there was significant and prolonged tumor PD correlating with enhanced tumor PK. This suggests that anti-tumor efficacy is model specific and a combination strategy in the BT-474 model may be advantageous.

Conclusions: BIND-2206 Accurins showed differentiated PK, increased tumor exposure, prolonged duration of target inhibition, superior efficacy and enhanced tolerability compared to parent MK-2206. These data suggest that nanoparticle formulations of the AKT inhibitor, MK-2206, may provide improved tolerability and therapeutic efficacy in a clinical setting.

C198 PDK1 inhibitors SNS-229 and SNS-510 cause pathway modulation, apoptosis and tumor regression in hematologic cancer models in addition to solid tumors. Stig Hansen1, Johan Enquist1, Jeff Iwig1, Minke E. Binnerts2, Gene Jamieson2, Judith A. Fox2, Adam R. Craig2. 1Carmot Therapeutics, San Francisco, CA; 2Sunesis Pharmaceuticals, Inc., South San Francisco, CA.

Background and purpose: Phosphatidyl-inositol (PI) dependent kinase 1, PDK1, is a master kinase that activates kinases important in cell growth and survival including members of the AKT, PKC, RSK and SGK families. PDK1 can interact with its substrates through PI-dependent (PH-mediated) or PI-independent (PIF-mediated) mechanisms. Here we report characterization of two potent PDK1 kinase inhibitors, SNS-229 and SNS-510, that block both PI-dependent and PI-independent substrate phosphorylation and have broad anti-tumor activity in hematologic cancers. Methods and results: SNS-229 and SNS-510 belong to a series of novel PDK1 inhibitors that bind the inactive conformation of PDK1 as determined by X-ray crystallography. The compounds bind deep in the adaptive pocket, distorting the N-terminal domain and perturbing the PIF-pocket, thereby affecting PI-independent substrate binding. SNS-510 was evaluated in more than 20 cell lines derived from hematologic cancers including AML, MM, DLBCL, and MCL and showed strong anti-proliferative activity with EC50s ranging from 3 nM to 900 nM, with particularly strong activity observed in the AML cell lines Molm-13 and MV4-11 (EC50 3 and 7 nM), the DLBCL cell line U-2932 (EC50 56 nM) and the MM cell lines U-266 and RPMI-8226 (EC50 130 and 163 nM). Anti-proliferative activity correlated with pathway modulation as assessed by inhibition of phosphorylation of PDK1, RSK, and AKT. Interestingly, inhibition of PDK1 phosphorylation was time-dependent showing 2 to 5-fold more inhibition after 24 hours than at 4 hours. In addition, SNS-510 produced substantial apoptosis after 24 hours. SNS-510 was compared to the PDK1 inhibitor GSK2334470, showing comparable biochemical potency. However, SNS-510 was 10 to 30 fold more potent at inhibiting PDK1 and RSK phosphorylation in all cell lines tested. SNS-510 was at least 10-fold more potent than GSK2334470 in 72 hour viability assays. In mice, SNS-229 and SNS-510 showed good oral bioavailability (%F>40%) with a Tmax of 4 to 8 hours and prolonged exposure. Pathway modulation was evaluated in vivo in a MV4-11 xenograft mouse model. Potent, dose-dependent pathway modulation was observed at 4 and 24 hours after a single oral dose of SNS-229 and SNS-510 (1 to 25 mg/kg). After 21-day dosing in MV4-11 xenografts, both SNS-229 and SNS-510 showed dose-related efficacy with > 95% tumor growth inhibition and partial regression (>50% tumor shrinkage) in 70% and 100% of animals at the highest dose. Conclusion: With this class of PDK1 inhibitors, we have previously reported strong tumor growth inhibition (66%-95%) in gastric, lung, pancreatic and colorectal cancer xenograft models. Here we report a PK/PD (pathway modulation) relationship that correlates with profound tumor growth inhibition in hematologic cancers. Thus, targeting the inactive conformation of PDK1 and inhibiting PI-independent substrate binding has broad potential for the treatment of solid and hematologic cancers.
C199  PI3K/AKT/mTOR inhibitors enhance the anti-tumor activity of the FGFR, VEGFR and PDGFR inhibitor lucitanib in FGF-dependent breast cancer models. Minh Nguyen, Liliane Robillard, Andrew D. Simmons, Henry J. Haringsma, Thomas C. Harding. Clovis Oncology, San Francisco, CA.

Lucitanib (S 80881, E-3810, CO-3810) is a potent inhibitor of the fibroblast growth factor receptors 1-3 (FGFR1-3), vascular endothelial growth factor receptors 1-3 (VEGFR1-3) and platelet-derived growth factor receptors alpha and beta (PDGFRα/β). A Phase 1/2 clinical study (Soria et al., 2014) of lucitanib showed a RECIST response rate of 50% in breast cancer patients with FGF-aberrant (FGFR1 and/or FGFR3/4/19 gene amplified) tumors. Here we explored potential drug partners that can be used in combination with lucitanib to increase cell cytotoxicity. Five FGF-dependent breast carcinoma cell lines were evaluated - MFM-223 (human TNBC, FGFR1/FGFR2 amplified, PIK3CA H1047R); HCC38 (human TNBC, FGFR1 high expression, PIK3CA W386L); CAL-120 (human TNBC, FGFR1/FRS2 amplified, PIK3CA wild-type); EFM-19 (human ER+ BC, FGFR2 K659E, PIK3CA H1047L); and 4T1 (mouse TNBC, FGFR2 high expression, PIK3CA wild-type). Single agent and lucitanib drug-drug combinations were evaluated in 2D monolayer and/or 3D spheroid culture using a broad (MFM-223; n=350 compounds) or a more focused compound library (cell lines CAL-120, EFM-19 and MFM-223; n=70 compounds approved or in clinical trials). Drug combinations were evaluated at a concentration range of 0.06-5000 nM and co-cultured with 0.2 µM lucitanib in a 72-hour cell viability assay. Combination efficacy was determined by comparing cell viability with and without lucitanib either by direct comparison of raw RLU or by fold change in calculated GI50. Several compounds were identified that enhanced cell killing when combined with lucitanib, including HDAC inhibitors (vorinostat, entinostat and belinostat), PI3K/Akt/mTOR pathway inhibitors (GDC-0941, GDC-0980 and BLY719), EGFR/HER2 inhibitors (afatinib and lapatinib), and CDK inhibitors (AT7519 and flavopiridol). These combinations were examined for efficacy across all five cell lines, and combination indexes (CI) were calculated to determine synergy. The PI3K/Akt/mTOR and lucitanib combination demonstrated consistent synergy across all cell lines independent of PIK3CA mutation status. For example, the PI3K inhibitor GDC-0941 showed a CI range of 0.04-0.58 at the GI50 (CI < 1 synergistic). Greater cell killing by combined PI3K/AKT and FGFR inhibition was also shown to correlate with increased inhibition of p-AKT by immunoblotting. In conclusion, the activity of lucitanib in FGF-driven breast carcinoma cells is augmented by the addition of PI3K/AKT/mTOR inhibitors, and dual targeting of AKT and FGFR signaling may enhance the clinical activity of these agents.

C200  Effects of PI3K/Akt pathway inhibition on global proteome levels and phosphorylation signaling in patient-derived xenograft models of triple negative breast cancer. Filip O. Mundt1, Cynthia Ma2, Philipp Mertins1, Zhanfang Guo1, Matthew Meyer2, Jana Qiao1, DR Mani1, Karl Clauser1, Shunqiang Li2, Kuan Huang3, Jason Held2, Sherri Davies2, Kelly Ruggles2, Li Ding2, Michael Gillette1, David Fenyo3, Reid Townsend2, Matthew Ellis4, Steven A. Carr1. 1Broad Institute, Cambridge, MA; 2Washington University, St. Louis, MO; 3New York University, New York, NY; 4Baylor College of Medicine, Houston, TX. *equal contribution.

Aim: The aim of this study was to identify markers that have the potential to predict BKM120 (a pan-class I PI3K inhibitor) effectiveness in triple negative breast cancer.

Introduction: Triple negative breast cancer is an aggressive subtype that constitutes circa 20% of all breast cancers. This subtype of breast cancer is defined by its lack of estrogen-, progesterone- and HER2/neu expression. Since these are the operative receptors for targeted breast cancer therapies, current standard treatment instead centers on conventional cytotoxic chemotherapy. Unfortunately, the majority of triple negative breast cancer patients does not have meaningful response, and new therapeutic agents are
needed. The PI3K/Akt/mTOR axis is often dysregulated in breast cancer, and clinical trials are ongoing for several inhibitors targeting this pathway. Differential analysis of the global proteome and phosphoproteome of BKM120-treated responsive and resistant tumors may identify markers, patterns and pathways associated with clinical response to support precision therapy.

Materials and Methods: We collected tumor material from triple negative breast cancer xenografts propagated in the mammary fat pads of NOD SCID mice after BKM120 treatment. Long term (~30 days) treatment was performed to assess growth rates and sensitivity to the drug. For mass spectrometry analyses, tumor material from 6 different mouse models across 5 treatment conditions was lysed and digested into peptides. Samples were labeled with TMT6-plex reagents, and analyzed together with one reference sample in a total of 6 TMT6-plex experiments. To increase proteome coverage, sample complexity was reduced using high-pH reversed-phase separation, and phospho-peptides were enriched using immobilized metal affinity chromatography. All samples were analyzed on a Q Exactive mass spectrometer.

Results and Data Analyses: Cumulatively, >19,000 proteins were identified, with an average of >10,000 proteins and >21,000 phosphosites per sample. The 6 mice showed a continuum of response to the drug from highly sensitive to highly resistant. Pearson correlation analysis was used to identify proteins and phosphosites that correlate to this response gradient, while moderate t-tests were used to identify commonly regulated markers. Preliminary analyses indicate several interesting proteins and phospho-sites, some pertaining to the PI3K/Akt pathway. Further canonical pathways were detected using Gene Set Enrichment Analysis on a sample-by-sample basis and additional network and pathway analyses were performed using the network analysis program GeNets, as well as Ingenuity Pathway Analysis.

C201 Activity of the GlaxoSmithKline kinase inhibitor set on glioblastoma stem cells. Simon Khagi1, Sejuti Sengupta2, Tate Tabtieng2, Brent H. Cochran2. 1Duke University Medical Center, Durham, NC; 2Tufts University, Boston, MA.

Despite significant advances in our understanding glioblastoma (GBM) in recent years, it remains a deadly cancer with a very poor prognosis. There is an urgent need for new therapies. Kinases are well known regulators of cellular growth and survival and there are many compounds available that are capable of inhibiting specific kinases. Recently, GlaxoSmithKline has made available a library of over 350 kinase inhibitors in a public-private partnership to facilitate the discovery of new drugs and targets in multiple disease settings (Nature Chemical Biology 9:3 (2013)). Together these compounds have been shown to inhibit over 100 different kinases by in vitro kinase assay at 1 uM. We have screened these compounds against 3 different GBM stem cell lines under normoxic and hypoxic conditions. Over 40 compounds were strongly inhibitory for least one cell line and condition. However, only 9 of these compounds were capable of inhibiting the growth all three cell lines by 90% at 1 uM in both conditions. Seven of the 9 common inhibitors had IC50’s under 100 nM and 4 had IC50’s under 10 nM in at least one oxygen condition. In vitro kinase assays indicate that these compounds each have multiple kinase targets. Based on an analysis of common targets, it is likely that one of the targets is PLK1 which is well known GBM inhibitor. Two compounds that don’t inhibit PLK1 both inhibit CLK2 and KIT strongly. IC50 analysis demonstrated wide variability among cell lines and oxygen concentrations. The effectiveness of a single compound could vary more then tenfold between cell lines and more than fiftyfold between normoxia and hypoxia. Some compounds were more effective under hypoxia and some were less. These results strengthen the case for personalized therapies and indicate that hypoxia (which is prevalent in GBM) can significantly alter the effectiveness of inhibitors.

The group of transcriptional regulatory proteins known collectively as the super enhancer complex (SEC) coordinate the expression of entire genetic programs directing cell fate. The SEC is also important in driving cancer progression mediated by transcription of key oncogenes such as c-Myc and Bcl-2. The SEC requires the interaction and coordination of many proteins, including cyclin-dependent kinases (CDK), bromodomain proteins (BRD), histone deacetylases (HDAC), and histone methyltransferases (HMT). Each of these proteins are the focus of significant development efforts for the treatment of cancer. SEC-regulated transcription requires recruitment of CDK9/cyclin T1 from the 7SK RNA/Hexim1 inhibitory complex by BRD4 to transcriptional start sites. CDK9 then phosphorylates RNA polymerase II, releasing it from the start site leading to productive transcriptional elongation and gene expression. Considering the close association of CDK9 and BRD4, we hypothesized that the combination of CDK9 and BRD4 inhibitors would have synergistic effects in cancer cells. Alvocidib is a potent CDK9 inhibitor with validated clinical activity in AML from multiple Phase II studies in over 400 patients. Additionally, BRD4 inhibitors have demonstrated early promise in clinical studies with a focus on hematologic malignancies. However, we have found that CDK9 inhibitors, combined with bromodomain inhibitors, produced a synergistic effect by inhibiting the SEC more effectively than either of these compounds alone. Cell viability studies with various combinations resulted in an increase in potency. This was observed with alvocidib combined with JQ-1 (BRD4 inhibitor) in A549 lung cancer cells. Furthermore, the combination of alvocidib with JQ-1 completely abrogated SEC function, as measured by c-Myc or Mcl-1 expression through RT-qPCR. Similar results were achieved with other combinations of CDK9 and BRD4 inhibitors. These data, primarily focused on alvocidib and JQ-1, suggest a strong rationale for combining CDK9 and BRD4 inhibitors as a treatment strategy for multiple tumor types, including lung cancer. Furthermore, these findings may be more broadly applied to additional therapeutic targets in the SEC. These strategies yield synergistic effects at inhibiting SEC function and are highly active in tumor growth studies of cancer, in vivo. Clinical studies utilizing these combination strategies will explore this therapeutic approach.

Tumor Suppressors

C203  Cracking the (ultra)structural biology of Scribble to understand its role as a cell polarity regulator and tumor suppressor protein. Krystle YB Lim¹, Sofia Cariá¹, Colin M. House², Nathan J. Godde², Allison J. Ogden², Patrick O. Humbert², Marc Kvansakul¹. ¹La Trobe Institute for Molecular Science, Melbourne, Australia; ²Peter MacCallum Cancer Centre, Melbourne, Australia.

Cell polarity or cell asymmetry is crucial for the normal biological function of all metazoan cells. Loss of cell polarity disrupts cellular organization that represents one of the hallmarks of cancer progression. Scribble, a member of the core polarity SCRIB module is deregulated in many cancers such as breast cancer, cervical cancer and prostate cancer. Scribble is a member of the LAP family that consists of 16 Leucine Rich Repeats and 4 PSD-95/Discs-large/ZO-1 (PDZ) domains. The PDZ domains (4PDZ) in particular are important in Scribble’s interactions with molecules that are involved in many crucial signaling pathways. We hypothesized that Scribble acts as a scaffold protein to orchestrate these interactions. Our aim is to decipher Scribble’s mechanism of action through characterization of Scribble interactions with βPIX (PAK-interacting exchange factor beta), which is well establish in exocytosis and neuronal transmission. Herein, we used pull down assay, isothermal titration calorimetry and small angle X-ray scattering to unravel the interaction
profile of the region of Scribble encoding for all four PDZ domains. Our study proposes a working model to illustrate how PDZ domains modulate Scribble and βPIX interactions.

C204 Characterization of TP53 mutations by DNA and RNA sequencing of platin-resistant ovarian cancer FFPE biopsies. Brian C. Haynes1, Diane Ilsley1, Marie Fahey1, Gary J. Latham1, Elizabeth B. Somers2, Daniel J. O’Shannessy2. 1Asuragen, Inc., Austin, TX; 2Morphotek, Inc., Exton, PA.

Introduction: TP53 is the most frequently mutated gene across all cancers at approximately 50% and in cancers such as high grade serous ovarian up to 96%. In addition to the high incidence of DNA mutations at the gene locus, the TP53 pathway is also dysregulated through alternative mechanisms which involve other members of the regulatory cascade such as overexpression of Mdm2 or Mdmx.

In contrast to other tumor-suppressors, TP53 possesses a spectrum of mutations including both loss and gain of function. Some mutations such as E177R result in a partial loss of TP53 function, eliminating the apoptotic fate while retaining senescence and cell-cycle arrest. Despite the centrality of TP53 in cancer, its prognostic significance remains unclear and the development of targeted therapies is ongoing. Characterizing TP53 mutation status in conjunction with transcripotional profiling of tumor biopsies will enable a more comprehensive understanding of the functional consequences of different classes of TP53 mutations and aid in distinguishing partial from total abrogation of TP53 pathway function.

Asuragen has developed sensitive assays and algorithms to characterize DNA mutations and RNA expression profiles from the most challenging poor quality clinical samples. In order to enable a more complete understanding of the functional consequences of TP53 mutations (or lack thereof), we present an application of these approaches to characterize TP53 function in FFPE ovarian cancer biopsies through an integrated analysis of DNA and RNA sequencing.

Methods: Primary FFPE tumor biopsies were collected from a cohort of 200 paclitaxel/carboplatin resistant ovarian cancer patients. DNA from isolated tumor biopsies and matched normal specimens was profiled by the Quantidex™ NGS TP53 Assay (Asuragen, Inc.) and library analysis was performed using the Quantidex™ Reporter. TP53 mutation status was independently assessed by the AmpliSeq™ Cancer Hotspot Panel (Thermo Fisher Scientific). Whole transcriptome RNA-Seq was performed on isolated total RNA material. Gene and isoform expression quantitation as well as SNV and indel calling was performed on the RNA-Seq libraries.

Results: TP53 mutation status for Quantidex TP53 and AmpliSeq assays was found to be concordant for regions of TP53 covered by both assays. The Quantidex TP53 Assay has more complete coverage of COSMIC mutations, 99% compared to 68% for AmpliSeq. Many TP53 positives were detected by the Quantidex Assay but negative by AmpliSeq due to coverage differences. The remaining discordant calls in commonly covered regions were assessed with matched RNA-Seq data. The comparison with RNA-Seq, led to the observation of specific cases of allele specific preferential expression of TP53 gain of function mutations. RNA-Seq expression profiles were also evaluated leading to the identification of gene signatures that associate with different TP53 mutation classes.

Conclusions: TP53 profiling through orthogonal DNA-Seq assays and matched RNA-Seq has enabled the identification of a set of high confidence associations between TP53 mutations and RNA expression signatures in ovarian cancer. These results point to a path for clinical assays in which DNA mutations and pathway signatures are integrated in support of diagnostic and precision medicine applications.
CD137 is a potent costimulatory immunoreceptor and a member of the TNF-receptor (TNFR) superfamily. The receptor, also known as 4-1BB, is mainly expressed on activated CD4+ and CD8+ T cells, activated B cells, and natural killer (NK) cells. While multiple lines of evidence show that CD137 is a highly promising therapeutic target, current approaches using monospecific antibodies may display a limited therapeutic window due to peripheral T cell and NK cell activation, leading to unwanted toxicity. To overcome this limitation, we have generated a bispecific protein therapeutic designed to achieve a tumor-target driven activation of immune cells via binding to CD137 and to a differentially expressed tumor target, HER2.

Anticalin® proteins are 18 kD protein therapeutics derived from human lipocalins. Using phage display technology a CD137-specific Anticalin was identified. The Anticalin was recombinantly fused to a trastuzumab variant at either the C or N terminus of the antibody’s heavy or light chain, yielding four different constructs covering a range of distances between the binding sites of the T cell-target and the tumor cell target. To minimize Fcγ-receptor interaction of the resulting bispecific and concomitant potential toxicity towards CD137-positive cells, the backbone of trastuzumab was switched from IgG1 to an engineered IgG4 isotype.

Using ELISA or cell-based assays it was shown that all bispecific constructs bound their targets CD137 and HER2 with similar affinity compared to the parental building blocks, and both targets could be simultaneously bound. Binding to human receptors FcγRI and FcγRIII was significantly reduced in the bispecific constructs compared to non-engineered trastuzumab, while binding to the neonatal Fc receptor (FcRn) was retained. All constructs were shown to have excellent drug-like properties including thermal stability and plasma stability. HER2-dependent agonistic engagement of CD137 was demonstrated in ex-vivo T-cell activation assays utilizing HER2-positive human cell lines. The functional activity of the bispecific constructs was found to be dependent on their geometry.

In conclusion, we report the first bispecific therapeutic protein that targets the potent costimulatory immunoreceptor CD137 in a tumor-target dependent manner, utilizing HER2 as the tumor target. Compared to currently existing CD137-targeting antibodies, this approach has the potential to provide a more controlled activation of the immune system in the tumor microenvironment with reduced peripheral toxicity. Bispecific T-cell engagers based on CD137 and HER2 have potential utility in HER2-positive cancers where there is a significant unmet medical need.