Oral Presentations from Proffered Abstracts

PR-001 *Ex vivo* co-culture system with patient-derived organoids to assess CXCR4 inhibitor as an immune modulating agent for human pancreas adenocarcinoma. Emily Alouani[1], Ilenia Pellicciotta[1], Winston Wong[1], Alexander S. Thomas[2], Michael D. Kluger[2], Anna M. Chiarella[1], Anil K. Rustgi[1], Gulam A. Manji[1]. [1]Columbia University Irving Medical Center, New York, NY, [2]Columbia University, New York, NY.

Introduction: Immune checkpoint blockade either alone or in combination with chemotherapy has been ineffective in pancreatic ductal adenocarcinoma (PDAC) likely due to underlying immunosuppressive pathways. The C-X-C chemokine receptor type 4 (CXCR4) / chemokine (C-X-C motif) ligand 12 (CXCL12) axis is a key immunosuppressive pathway where CXCL12 released by cancer-associated fibroblasts (CAF) binds to its receptor CXCR4 expressed by cytotoxic $CD8^+$ T cells (CTL) which results in their decreased abundance within tumors. Encouraging preclinical and clinical data from mouse PDAC models and human PDAC tumor sections have shown T cell tumor accumulation after co-inhibition of CXCR4 and PD1/PDL1, however number of tumor regressions were limited. **Results:** To further explore the mechanism by which inhibition of the CXCR4/CXCL12 pathway modulates the immune TME in triggering an anti-tumor immune response in human PDAC, we generated an autologous co-culture system with patient-derived tumor organoids (PDTO) and peripheral blood mononuclear cells (PBMC). PDAC tumor tissue from surgical resection was used to generate lines of PDTO that recapitulate the histological and genetic characteristics of the original tumor. We demonstrate that tumor-specific reactive T cells can be obtained in PDAC by co-culture with autologous PDTO, with up to 5% of $CD8^+$ T cells producing IFNγ after tumor organoid stimulation. No T cell reactivity was observed against normal pancreatic organoids, and blocking tumor MHC I/MHC II decreased the proportion of activated T cells. *Ex vivo* migration assays were established to better understand the role of CXCL12 in PDAC tumor T cell exclusion. Tumor organoids attracted autologous PBMC and addition of recombinant CXCL12 decreased PBMC migration, while treatment of PBMC with the CXCR4 inhibitor AMD3100, rescued the number of PBMCs that were able to migrate towards the tumor organoids. PBMC infiltrated PDTO-containing but not empty Matrigel domes. Addition of AMD3100 increased PBMC infiltration within the Matrigel dome containing PDTOs. Finally, we found that pre-treatment of PBMC with AMD3100 increased T cell reactivity against PDTO, and that T cell activation was further increased when stimulated with PDTO pre-treated with chemotherapy. **Conclusions:** We found that CXCL12 modulates the migration of T cells towards tumor organoids generated from pancreatic cancer tissue, and that inhibition of CXCR4 not only increases the migration potential, but also increases tumor-specific T cell activation. The use of *ex vivo* autologous co-culture and migration assays provide a unique strategy to identify modulators that may enhance T cell mediated neoplastic cell death to guide therapeutic intervention in PDAC.

Early Phase Clinical Trials

PR-002 A phase II pilot trial of nivolumab (N) + albumin bound paclitaxel (AP) + paricalcitol (P) + cisplatin (C) + gemcitabine (G) (NAPPCG) in patients with previously untreated metastatic pancreatic ductal adenocarcinoma (PDAC). Erkut Borazanci[1], Gayle S. Jameson[1], Sunil Sharma[1], Frank Tsai[2], Ronald L. Korn[3], Lana Caldwell[2], Karen Ansaldo[2], David

T. Ting[4], Denise Roe[5], Anna Bermudez[2], Daniel D. Von Hoff[1]. [1]HonorHealth/TGen, Scottsdale, AZ, [2]HonorHealth, Scottsdale, AZ, [3]Imaging Endpoints, Scottsdale, AZ, [4]Massuchusetts General, Boston, MA, [5]University of Arizona, Tucson, AZ.

Background: Effective therapy for the treatment of PDAC remains one of the greatest unmet oncology clinical needs. The addition of C to G and AP has shown 71% ORR in a previously reported study [JAMA Oncol. 2019 Oct 3;6(1):125-32]. In preclinical work, vitamin D (Vit D) analog therapy decreases myeloid derived suppressor cells and regulatory T cells, turning PDAC into a more immune favorable microenvironment. This trial combines AP/C/G with Vit D analog P and the anti-PD-1 antibody N as a combination therapy for patients with previously untreated metastatic PDAC. This trial evaluates the efficacy and safety of NAPPCG in that patient population (NCT02754726). **Methods:** Eligibility criteria include Stage IV PDAC, no prior chemotherapy for systemic disease, KPS ≥ 70, and RECIST 1.1 measurable disease. Doses are AP 125 mg/m^2, G 1000 mg/m^2, each infused over 30 minutes with C 25 mg/ m^2 infused over 60 minutes on days 1, 8, 22, and 29 of a 42-day cycle. N is given at a fixed dose of 240 mg as a 60 minute infusion on days 1, 15, and 29. P is given at a fixed dose of 25 µg IV twice weekly. Primary objective was to determine the efficacy of the combination for patients with previously untreated metastatic PDAC through determining CR, ORR, PFS, and OS. The secondary objective was to evaluate safety in patients with previously untreated metastatic PDAC. Exploratory endpoints include evaluating tissue molecular profile as it relates to treatment outcomes. **Results:** Trial was conducted May 2016 with enrollment completed August 2020. 35 patients have been enrolled in the study and 32 are evaluable (baseline and ≥1 follow up CT scan). Most common drug-related grade (Gr) 3-4 adverse events (AE's), are thrombocytopenia 76% (gr 3 = 34%, gr 4 = 28%) with no serious bleeding events, anemia 37% (gr 3 = 37%, gr 4 = 0%), and CIPN 11% (gr 3 = 11%, gr 4 = 0%). Immune Related Adverse Events >5% were colitis (gr 3=8.6%, gr 4= 0%) and dermatitis (gr 3=8.6%, gr 4= 0%). By RECIST 1.1 criteria, the best response is 1 CR, 26 PR, 4 SD, 1 PD, yielding an 84% ORR (95% CI = (67%, 95%). Median PFS is 6 months (95% CI = (5, 8)). Median OS is 18 months (95% CI = (13, 22)). **Conclusions:** Although a small study, the high response rate is encouraging. Evaluation of exploratory endpoints is ongoing. Pursuing this regimen in localized PDAC is warranted due to its high ORR. Supported by grants from the Seena Magowitz Foundation, Mattress Firm, Bristol Myers Squibb, and SU2C.

Immunotherapy

PR-003 High quality neoantigens are immunoedited in long-term pancreatic cancer survivors. Luis A. Rojas[1], Marta Łuksza[2], Zachary M. Sethna[1], Kevin Soares[1], Joanne Leung[1], Jayon Lihm[1], David Hoyos[1], Anton Dobrin[1], Rajya Kappagantula[1], Alvin Makohon-Moore[1], Amber Johns[3], Anthony Gill[3], Masataka Amisaki[1], Pablo Guasp[1], Abderezak Zebboudj[1], Rebecca Yu[1], Adrienne Kaya Chandra[1], Zagaa Odgerel[1], Michel Sadelain[1], Erin Patterson[1], Christine Iacobuzio-Donahue[1], Benjamin D. Greenbaum[1], Vinod P. Balachandran[1]. [1]Memorial Sloan Kettering Cancer Center, New York, NY, [2]Icahn School of Medicine at Mount Sinai, New York, NY, [3]Garvan Institute of Medical Research, New South Wales, Australia.

Cancer immunoediting is a hallmark of cancer that predicts T cells recognize and kill tumor cells expressing immunogenic mutations (neoantigens), to induce less immunogenic clones to

outgrow in tumors. Although established through longitudinal studies of how tumors evolve in immune-proficient and -deficient mice, whether the human immune system naturally targets neoantigens to edit tumors remains unclear. Here, we investigate how 70 human pancreatic ductal adenocarcinomas (PDACs) – a poorly immunogenic cancer with few neoantigens and thus largely presumed to not be subject to immunoediting – evolved over 10 years. We use longitudinal tumor sampling to compare how primary tumors evolve to recurrence in rare long-term PDAC survivors previously shown to have more immunogenic tumors (n = 9 patients, n = 9 primary and 22 recurrent tumors, median survival 5.4 years), to short-term survivors with less immunogenic primary tumors (n = 6 patients, n = 6 primary and 33 recurrent tumors, median survival 1.8 years). Compared to short-term survivors, we observe that long-term survivors evolve fewer recurrent tumors with longer latency, and distinct tissue tropism. To evaluate if differential immune pressures could explain these differences, we perform whole exome sequencing to bioinformatically predict tumor clonal structures and neoantigens. We discover that despite longer times to evolve, long-term survivors evolve genetically less heterogeneous tumors with fewer clones, fewer nonsynonymous mutations, and fewer neoantigens. To identify the edited neoantigens, we sought to improve upon and apply our previously defined quality model that quantifies the immunogenic features of a neoantigen. With our quality model, we now infer a neoantigen is immunogenic ("high quality") by two parameters – if the immune system can recognize a neoantigen as "non-self" by its similarity to known immunogenic antigens, and discriminate it from "self" by estimating if a neoantigen has sufficient antigenic distance from its wild-type peptide to differentially bind the MHC or activate a T cell. We integrate these features to estimate neoantigen quality in primary and recurrent tumors of long- and short-term survivors. With the quality model, we observe that neoantigens with greater antigenic distance ("less self") are more depleted in primary and recurrent tumors of long- compared to short-term survivors. Furthermore, we find that long-term survivors evolve markedly fewer new neoantigens of strikingly lower quality, to indicate clones with high quality (more immunogenic) neoantigens are immunoedited. Thus, we submit longitudinal evidence that the human immune system naturally edits neoantigens in PDAC. Furthermore, we present a model that describes how cancer neoantigens evolve under immune pressure over time, with implications for cancer biology and therapy. More broadly, our results argue that immunoediting is a fundamental cancer suppressive mechanism that can be quantified to predict tumor evolution.

PR-004 Inhibition of focal adhesion kinase (FAK) improves pancreatic ductal adenocarcinoma's response to immunotherapy by targeting cancer stem cells (CSCs). Yezi Zhu[1], Lyndsey Sandow[2], William Matsui[1]. [1]LIVESTRONG Cancer Institute, Dell Medical School, UT Austin, Austin, TX, [2]Oregon Health & Science University, Portland, OR.

Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers with a 5-year survival rate less than 10%. Current therapies consisting of cytotoxic chemotherapeutic agents are not effective in most patients with metastatic PDAC, and the impact of new strategies, including immunotherapies, has not been established. Previous studies have shown that inhibition of focal adhesion kinase (FAK) modulates the PDAC immunosuppressive tumor microenvironment (TME), and sensitizes tumors to immune checkpoint blockade. However, the role of FAK in regulating tumor cell intrinsic resistance to immunotherapy is not well understood. We and others previously demonstrated FAK regulates PDAC cancer stem cells (CSCs) activity including self-renewal, tumor initiation, and drug resistance. These CSCs are

responsible for metastatic dissemination and resistance to multiple therapies, including immunotherapy. In this study, we evaluated the sensitivity of PDAC cells, including CSCs to cytotoxic T cells, and we also analyzed the impact of FAK inhibition on the immunogenicity and the response to cytotoxic T cell activity of PDAC CSCs. **Methods**: To understand how FAK regulates tumor intrinsic resistance to antigen-specific cytotoxic T cells, we utilized OT-I T cells (OT-I cells) that specifically recognize a peptide fragment of ovalbumin (ova) within the context of MHC-I. Mouse KPC pancreatic cancer cell lines expressing ova were generated and co-cultured with cytotoxic CD8+ OT-I cells in vitro. We analyzed the effect of FAK in regulating bulk cells sensitivity to antigen-specific cytotoxic T cells and clonogenic growth of CSCs by flow cytometry, MTT assay and colony formation assay using CRISPR-Cas9 and small molecule FAK inhibitors. We also analyzed the expression of surface proteins that mediate interactions with T cells (class I MHC) in bulk cells and CSCs after FAK inhibition. **Results**: Loss of FAK activity decreased CSCs activity (self-renewal, colony formation) in PDAC mouse cell lines, which is consistent with our previous data. Using antigen-specific OT-I co-culture model, we found that CSCs are resistant to cytotoxic T cells anti-tumor activity compared to bulk tumor cells. We also found inhibition of FAK activity not only enhances PDAC cells to cytotoxic T cell activity, but also sensitized CSCs to T cell killing. Studies in both human and mouse PDAC cell lines demonstrated decreased MHC-I expression in CSCs compared to the bulk tumor cells which could be reversed by FAK inhibition. **Conclusion**: We have demonstrated that CSCs are resistant to antigen-specific cytotoxic T cells, and FAK inhibition in CSCs can sensitize these cells to anti-tumor T cell activity that is associated with changes in the expression of factors involved in antigen presentation. Our study provides novel insights into the unique immunogenicity of CSCs and the role of CSCs in regulating tumor intrinsic immunotherapy resistance in PDAC. Our findings also lead to the identification of FAK as a novel therapeutic target for CSCs to improve immunotherapy efficacy in PDAC.

Big Data

PR-005 Chromatin dynamics *in vivo* define coordinate functions of inflammation and mutant Kras in pancreatic tumorigenesis. David Falvo[1], Jason Pitarresi[2], Alexa Osterhoudt[1], Adrien Grimont[1], Ben Stanger[2], Steven D. Leach[3], Anil K. Rustgi[4], Rohit Chandwani[1]. [1]Weill Cornell Medicine, New York, NY, [2]University of Pennsylvania, Philadelphia, PA, [3]Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, NH, [4]Columbia University Irving Medical Center, New York, NY.

Pancreatic cancer initiation features abundant rewiring of the normal acinar cell,. but little is known about the chromatin specification of pancreatic cell types and the epigenetic dysregulation of normal acinar cells in tumor initiation. To address these questions, we employed a lineage-traced autochthonous mouse model to examine systematically perturbed acinar cells. We coupled the spatiotemporal control of inflammation (via caerulein injections) with inducible oncogene activation (mutant Kras*)* in the adult mouse along with bulk RNA-seq and ATAC-seq to sorted acinar and acinar-derived cells. In addition, we generated Ptf1a-TdTomato mice to reliably sort pancreatic progenitors at e10.5 and e15.5. We observe that *Kras* activation alone does not disturb acinar cell chromatin nor the histologic appearance of the pancreas. By contrast, caerulein alters chromatin significantly in metaplasia and even in regeneration, with putative enhancers derepressed despite normal histology. In the context of *Kras* activation and caerulein

administration, we find a broad and stable reorganization of chromatin, reflecting cooperativity between oncogenic stress and an inflammatory insult. We also find that in PanIN, the chromatin state bears few, if any, ductal, progenitor, or islet features and instead reflects a largely novel cell fate. To understand the dependencies of these findings on an inflammatory insult and mutant Kras, we leveraged temporal resolution of pancreatitis and the iKras system to define the reversibility of this epigenetic rewiring. Notably, neither resolution of inflammation nor withdrawal of mutant Kras expression are sufficient to revert an acinar cell to its initial chromatin state. Analysis of the acinar-derived cells undergoing the transition to PanIN reveals the induction of specific proliferative and progenitor master transcription factors and activation of associated transcriptional programs. In these data we also observe a specific unveiling of the AP-1 isoform Fra-1 (Fosl1) transcript, protein, and binding sites in chromatin. To address if Fra-1-associated alterations to chromatin are bona fide requirements for tumorigenesis, we coupled conditional Fra-1 knockout alleles with the iKras system, finding nearly complete ablation of PanIN in the absence of Fra-1. Together, our findings suggest that (1) loss of acinar cell identity is resistant to oncogenic stress and is susceptible to inflammation; (2) the acquired acinar cell fate reflects neither 'pure' metaplasia nor transdifferentiation nor dedifferentiation events, and (3) acinar cell regeneration is incomplete. In contrast to recent studies, we demonstrate that pancreatic tumorigenesis does not re-establish a progenitor cell fate, but hijacks the AP-1 transcription factors for tumor-specific genomic locations, with Fra-1 emerging as a dependency in tumorigenesis. Our data thus highlight the complexity of cell fate decisions in the preneoplastic pancreas and reveal key regulators of acinar cell identity.

PR-006 Integrative genomic characterization of therapeutic targets for pancreatic cancer. Jimmy A. Guo[1], Daniel Zhao[2], Scott P. Ginebaugh[3], Steven Wang[4], Ananya D. Jambhale[1], Patrick Z. Yu[1], Westley W. Wu[1], Peter Chen[1], Maryann Zhao[1], Kristen E. Lowder[3], Kevin S. Kapner[3], Hannah I. Hoffman[1], Stephanie W. Cheng[5], Daniel Y. Kim[6], Rebecca Boiarsky[7], Francois Aguet[1], Brenton Paolella[1], John M. Krill-Burger[1], James M. McFarland[1], Tobiloba Oni[8], Tyler Jacks[7], Aviv Regev[9], Gad Getz[1], William L. Hwang[10], Harshabad Singh[3], Andrew J. Aguirre[3]. [1]Broad Institute of MIT and Harvard, Cambridge, MA, [2]New York Medical College, Valhalla, NY, [3]Dana Farber Cancer Institute, Boston, MA, [4]Columbia University, New York, NY, [5]Stanford University, Palo Alto, CA, [6]Harvard Medical School, Boston, MA, [7]MIT, Cambridge, MA, [8]Whitehead Institute, Cambridge, MA, [9]Genentech, San Francisco, CA, [10]Massachusetts General Hospital, Boston, MA.

Targeted therapies for molecularly-defined subtypes have led to immense clinical benefit for many cancer types but have generally not been successful for pancreatic cancer. Given that the mainstay of treatment remains multi-agent chemotherapy with FOLFIRINOX or gemcitabine/nab-paclitaxel, there remains an urgent need to identify novel actionable vulnerabilities for subsets of PDAC patients. Toward this end, we conducted an integrative, genome-scale examination of genetic dependencies and cell surface targets for PDAC by leveraging CRISPR and RNAi screening data from The Cancer Dependency Map Project, genomic data of bulk patient tumors from The Cancer Genome Atlas, and custom single-nucleus RNA-seq of a 43-patient cohort comprised of untreated and treated specimens. Our results re-affirm the prominence of Ras/MAPK signaling and a synthetically-lethal interaction between VPS4A/B, but also reveal recurrent susceptibilities to genes within the fatty acid metabolism, vesicular transport and exocytosis, and nucleobase synthesis pathways that otherwise have minor

to moderate depleting effects on the majority of cell lines. Aberrations in frequent tumor suppressor genes and chromosomal arm-level variations appear to modify the strength of dependencies, including that of *KRAS*, *CCND1*, and *GPX4*, and may serve as predictive biomarkers of response. In addition, we leveraged mRNA profiling of bulk primary tumors as well as metastatic organoid models to conduct a genome-wide search for cell surface targets that are highly-expressed in tumors while lowly or not expressed in other toxicity-prone, non-malignant tissues. These putative drug targets do not need to be cancer dependencies and can be compatible with antibody-based therapeutic strategies that leverage alternative modes of cellular toxicity. Our approach identifies MSLN, NECTIN4, TROP2, and other antigens which have previously been shown to be largely tumor-specific but also reveals the expression of multiple putative targets within the CEACAM, claudin, and tetraspanin families. Finally, molecular subtyping efforts over the past decade have yielded classical and basal-like as consensus subtypes with variations therein, but genetic dependencies and cell surface expression patterns unique to either are insufficiently understood. We identified *CLDN18*, *CEACAM5*, and *CEACAM6* as cell surface antigens for the classical subtype and *MSLN*, *AQP5*, and *SLC6A14* for basal-like. Dependency on *TLK2* and *CCND1* is associated with the basal-like and classical subtype, respectively. Taken together, our integrative genomic approach may provide a precision medicine blueprint for stratifying and targeting pancreatic cancer.

PR-007 Lung-tropic, liver-averse, primary PDAC tumors are associated with greater peripheral T cell diversity and have a unique, subtype-independent, gene-expression signature that significantly correlates with longer survival. Jason M. Link, Patrick J. Worth, Dove Keith, Sydney Owen, Alison Grossblatt-Wait, Carl Pelz, Hannah Holly, Motoyuki Tsuda, Kevin MacPherson, Jonathan Brody, Charles Lopez, Brett C. Sheppard, Rosalie C. Sears. Oregon Health & Science University, Portland, OR.

Pancreatic Ductal Adenocarcinoma (PDAC) is predicted to become the second leading cause of cancer-related death in the United States, and most patients who present with metastatic PDAC die within a year. However, we and others have found that patients with lung metastases in the absence of liver metastases survive significantly longer than patients who present with liver metastases. We analyzed an unpublished RNASeq dataset from ~300 tumor-enriched samples from primary and metastatic PDAC specimens. Consistent with many previous publications, we found that patients with basal/squamoid-subtype tumors had significantly worse outcomes than patients with classical/ductal-subtype tumors. Additionally, we identified that most primary tumors from patients who develop lung – but not liver – metastases are classical subtype. However, this association did not wholly account for the pro-survival effect of lung-tropic, liver-averse metastatic disease because patients with lung-tropic, liver-averse, classical-subtype, primary tumors had significantly better outcomes than patients with liver-tropic, classical-subtype tumors. To identify and parse metastatic organotropism from subtype, we used organotropism-independent and subtype-independent, primary-tumor training cohorts to generate two non-overlapping gene sets that were significantly enriched in test cohorts of either primary, basal-subtype or liver-tropic tumors over primary tumors that were classical-subtype or lung-tropic and liver-averse, respectively. When applied to all primary tumors in our dataset, both the subtype-specific and organotropism-specific gene sets significantly correlated with patient outcome. From an unpublished analysis of TCRbeta CDR3 sequences from ~250 paired blood and primary tumor samples, we identified significantly greater TCRbeta diversity in blood and

primary tumors from patients with lung-tropic, liver-averse disease. Additionally, we found evidence that TCRbeta rearrangements from liver-tropic primary tumors were more likely to be found in autologous peripheral blood samples than TCRbeta rearrangements from lung-tropic, liver-averse primary tumors. We also found that TCRbeta sequences were often shared between samples from patients with liver-tropic disease but never shared between samples from patients with lung-tropic, liver-averse disease. Overall, our results point to a lung-tropic, liver-averse form of PDAC that – independent of tumor subtype – leads to positive outcomes, and that T cell diversity may have a causal relationship and/or may serve as a biomarker of long-term survival with lung-tropic, liver-averse disease.

Signaling

PR-008 Kdm6 demethylases are critical regulators of pancreatic cancer initiation, progression and subtype specification. Laura Leonhardt, Lucia Y. Li, David I. Berrios, Sudipta Ashe, Audrey M. Hendley, Grace E. Kim, Matthias Hebrok. University of California San Francisco, San Francisco, CA.

Aberrant epigenetic regulation is a hallmark of pancreatic cancer. Since changes in histone methylation are potentially reversible, they present an attractive target for therapeutic intervention. Kdm6a and Kdm6b belong to a family of histone demethylases specific for lysine 27 of histone 3 and have been implicated in multiple types of cancer, including pancreatic ductal adenocarcinoma (PDA). We have gathered data from the extensive investigation of a mouse model of PDA in which either Kdm6b or both Kdm6a and Kdm6b have been deleted with concomitant activation of oncogenic $Kras^{G12D}$ restricted to adult acinar cells. While prior work had demonstrated an important role for Kdm6a in PDA formation, our data now show that the closely related Kdm6b is implicated in PDA tumorigenesis as well. Partial or complete loss of Kdm6b results in accelerated acinar-to-ductal metaplasia (ADM) and formation of pancreatic intraepithelial neoplasia (PanIN) with increased inflammation and fibrosis. Accelerated ADM formation was also observed *ex vivo* upon culturing acinar cells in the absence of oncogenic Kras, indicating that Kdm6b activity is critical for the maintenance of acinar identity and suppresses the early stages of pancreatic neoplasia development. However, only mice heterozygous for Kdm6b continue to exhibit a reduced overall median survival compared to $Kras^{G12D}$ alone, while complete loss of Kdm6b results in a similar overall median survival compared to $Kras^{G12D}$. This suggests a requirement for oncogenic Kdm6b function during later progression of PanIN to invasive cancer. Conversely, concomitant loss of Kdm6a and Kdm6b protects against ADM and PanIN formation *in vivo* and *ex vivo* and double mutants display a trend towards an increased overall median survival compared to mice carrying only the $Kras^{G12D}$ mutation. Strikingly, the resulting tumors of Kdm6a/Kdm6b deficient mice exhibit features of an extremely rare subtype of sarcomatoid pancreatic cancer found in human patients. Taken together, this suggests that Kdm6 demethylase function is required during the initiation and progression of PDA. Furthermore, targeting Kdm6a/Kdm6b with specific inhibitors at distinct stages might provide opportunities for novel treatment avenues for pancreatic cancer.

Metabolism

PR-009 Targeting the sterol regulatory element-binding protein pathway in pancreatic ductal adenocarcinoma. Stephanie Myers, Meredith McGuire, Wei Shao, Chine Liu, Theodore Ewachiw, Zeshaan Rasheed, William Matsui, Toni Sepalla, Richard Burkhart, Peter Espenshade. Johns Hopkins University, School of Medicine, Baltimore, MD.

Background: Pancreatic ductal adenocarcinoma (PDAC) is a very aggressive tumor with limited diagnostic and therapeutic options. Due to its proliferative nature and thick, desmoplastic stroma, PDAC tumor cells are challenged with meeting a high demand for lipids in a hypoxic, lipid-poor environment. Cancer cells respond to lipid conditions through sterol regulatory element-binding proteins (SREBPs). SREBPs are master transcriptional regulators of lipid homeostasis and require SREBP cleavage activating protein (SCAP) during signaling. While the SREBP pathway has been implicated in several cancers, its role in PDAC has not been examined. In this study, we assess the requirement of this pathway using both *in vitro* and *in vivo* model systems. Furthermore, we target the SREBP pathway using a novel combination therapy of two FDA-approved compounds: dipyridamole and statins. **Methods:** We acquired four patient-derived pancreatic adenocarcinoma cell lines containing mutations in both *Kras* and *p53* and knocked out *SCAP* in each cell line followed by gene-based rescue. Functional growth assays were performed in both lipid-poor and lipid-rich media conditions. Subcutaneous and pancreatic orthotopic xenografts were performed in nude mice using these same cell lines. A previously established mouse line, *LSL-Kras*$^{G12D/+}$*; LSL-Trp53*$^{R172H/+}$; *Pdx-1 Cre* (KPC) on a C57Bl/6 background, was utilized as a PDAC model. KPC mice lacking *Scap* in one or both alleles ($S^{fl/+}$ or $S^{fl/fl}$) were generated. Cohort survival and histologic analysis were performed for all mice, and results plotted on a Kaplan-Meier survival curve. In each patient cell line, the following drugs were applied individually and in combination: Dipyridamole (DP), tetramethyl-DP (TMDP), Fluvastatin, and Simvastatin. Cell viability post-treatment was assessed, and synergy calculated for each combination using SynergyFinder. **Results:** In lipid-poor conditions, *SCAP* knockout cells showed significantly reduced growth when compared to wild type or *SCAP* rescued cells. In tumor xenograft models, *SCAP* knockout cells exhibited reduced tumor growth and tumor volume when compared to wild-type cells. In the genetically engineered mouse models, KPC mice exhibited a median survival time of 289 days, while $KPCS^{fl/+}$ mice show a significantly increased median survival time of 425 days. Additionally, $KPCS^{fl/fl}$ mice did not develop invasive PDAC. Cells treated individually with DP, TMDP and both statins exhibit lipid-dependent growth defects in all cell lines tested. In combination, DP with either statin as well as TMDP with either statin demonstrate synergy in lipid-poor conditions. **Conclusions:** Our results demonstrate that loss of *SCAP* in PDAC tumor cells alters the growth capability both *in vitro* and using mouse xenograft models. Additionally, heterozygous loss of *Scap* in the KPC mouse model significantly increased survival. Finally, dipyridamole works in synergy with statins to alter growth of PDAC tumor cells. These findings suggest that targeting the SREBP pathway has significant therapeutic potential in PDAC.

Signaling

PR-010 Collateral amplification of the *KRAS* linked gene *PTHLH* governs pancreatic cancer growth and metastasis and reveals a new therapeutic vulnerability. Jason R. Pitarresi[1], Robert J. Norgard[1], Anna M. Chiarella[2], Kensuke Suzuki[2], Richard Kremer[3], Ben Z. Stanger[1], Anil K. Rustgi[2]. [1]University of Pennsylvania, Philadelphia, PA, [2]Columbia University,

New York, NY, [3]McGill University, Montreal, QC, Canada.

Purpose: Metastasis is the leading cause of cancer-related death in PDAC, yet very little is understood regarding the underlying biology. As a result, targeted therapies to inhibit metastasis are lacking. Whole-genome sequencing has established that the squamous/quasi-mesenchymal/basal-like PDAC subtype, which is characterized by its high metastatic proclivity, is annotated by *KRAS* gene amplification. Here, we report that the squamous lineage gene parathyroid hormone-related protein (PTHrP encoded by *PTHLH*) is located directly adjacent to *KRAS* and is co-amplified in metastatic PDAC patients. We hypothesize that this collateral amplification of PTHrP may exert its own oncogenic and pro-metastatic phenotype beyond KRAS and set out to determine if this will confer a novel therapeutic vulnerability. **Methods:** We generated a novel genetically engineered mouse model whereby we deleted the cytokine *Pthlh* in the autochthonous KPCY model. To functionally demonstrate the oncogenic and pro-metastatic roles of PTHrP, we further employed genetic deletion and pharmacological inhibition in orthotopic injection, tail vein metastasis assays, mouse hospital pre-clinical trials, and patient-derived 3D organoid models. **Results:** *In silico* analysis established that *PTHLH* is co-amplified along with *KRAS* in TCGA, is specifically enriched in metastatic patients from the COMPASS trial, and correlates with significantly decreased overall survival in both cohorts. Further examination revealed that *PTHLH* is a squamous/quasi-mesenchymal/basal-like lineage marker. We generated KPCY-Pthlh^{CKO} mice and showed that they have significantly reduced primary and metastatic tumor burden and dramatically increased overall survival relative to KPCY controls. In parallel experiments, we treated mice with an anti-PTHrP neutralizing monoclonal antibody, which similarly reduced primary and metastatic tumor growth. Finally, RNA-seq revealed a downstream mechanism whereby PTHrP is important for metastatic competency through induction of EMT, thus facilitating entry into the metastatic cascade. Loss of PTHrP reduced the ability of tumor cells to undergo EMT both *in vivo* and *in vitro*, resulting in a nearly complete elimination of disseminating cells in KPCY-Pthlh^{CKO} mice. Thus, KPCY-Pthlh^{CKO} tumors are locked in a well-differentiated epithelial state and are unable to initiate the metastatic process. **Conclusions:** This work has demonstrated the importance of the previously unappreciated role for PTHrP signaling in pancreatic cancer cell plasticity and metastasis, and future studies will look to translate anti-PTHrP therapy into clinical trials. In a broader sense, we establish a new paradigm of collateral amplification, where an assumed passenger gene (*PTHLH*) is co-amplified along with a known oncogene (*KRAS*) and endows the evolving tumor with its own oncogenic and pro-metastatic phenotype.

PR-011 Loss of compensatory feedback mechanism involving splicing factor SRSF1 accelerates *Kras^{G12D}*-mediated pancreatic cancer initiation. Ledong Wan, Kuan-Ting Lin, Mohammad A. Rahman, Zhikai Wang, Youngkyu Park, David A. Tuveson, Adrian R. Krainer. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

KRAS is recurrently mutated in pancreatic ductal adenocarcinoma (PDAC), triggering the formation of acinar-to-ductal metaplasia (ADM) and pancreatic intraepithelial neoplasia (PanIN). However, the majority of pancreatic cells from KC (*LSL-Kras$^{G12D/+}$; Pdx-1-Cre*) mice carrying the *Kras^{G12D}* mutation remain morphologically normal for a long time, suggesting the existence of compensatory feedback mechanisms that buffer *Kras^{G12D}* signaling, and that additional steps are required for disrupting cell homeostasis and promoting transformation. Here we found that

splicing factor SRSF1, which is associated with cell transformation, is downregulated in the majority of morphologically normal pancreas cells with the *Kras*^{G12D} mutation as a compensatory feedback mechanism. To assess the role of SRSF1 in PDAC, we generated a transgenic mouse strain (**SC**) with Dox-inducible pancreatic-specific expression of SRSF1 protein. Elevated SRSF1 alone is sufficient to induce pancreatitis and ADM transformation. By further crossing the SC strain with *Kras*^{G12D} alone (**KSC**) or together with *Trp53*^{R172H} (**KPSC**), we found that increasing SRSF1 accelerated *Kras*^{G12D}-mediated tumor initiation and resulted in more aggressive PDAC. To address the underlying mechanisms of SRSF1's involvement in the homeostatic response to *Kras*^{G12D} mutation and in PDAC initiation, we generated organoid lines from the above mouse strains. Following Dox induction, elevated SRSF1 with *Kras* WT or *Kras*^{G12D} consistently activated MAPK signaling, which is one of the top negatively enriched pathways in response to *Kras*^{G12D}. Furthermore, SRSF1 promoted the expression of *Il1r1*—an IL1 receptor associated with the activation of MAPK signaling—by regulating an alternative-splicing event in the 5'UTR to generate a more stable mRNA isoform. We conclude that decreased SRSF1 is a compensatory feedback mechanism in pancreatic cells against the *Kras*^{G12D} mutation, whose disruption facilitates PDAC initiation.

Tumor Microenvironment

PR-013 The splanchnic mesenchyme during fetal development is the major source of pancreatic cancer associated fibroblasts. Lu Han[1], Yongxia Wu[1], Melodie Parrish[1], Khushbu Patel[1], Xuezhong Yu[1], Michael Ostrowski[1], Gustavo Leone[2]. [1]Medical University of South Carolina, Charleston, SC, [2]Medical College of Wisconsin, Milwaukee, WI.

In pancreatic ductal adenocarcinoma (PDAC), cancer associated fibroblasts (CAFs) play critical and complex roles in the tumor microenvironment. CAFs are also a major cell type in the desmoplastic stroma in PDAC and may account for half of the entire tumor tissue. Multiple subtypes of CAFs have been suggested, but the tissue origin(s) of CAF subtypes are unknown and genetic tools to robustly target them *in vivo* are lacking. Here we aimed to examine three potential tissue sources of CAFs: the pancreatic epithelium (through epithelium-to-mesenchyme transition), the bone marrow (through circulation), and the pancreatic mesenchyme or tissue resident fibroblasts (TRFs) in the normal pancreas (through proliferation). We utilized a genetically engineered mouse model of PDAC, where *Kras* and *p53* mutations were engineered in the pancreatic epithelium using an *Flp-Frt* system. To determine whether the pancreatic epithelium gives rise to CAFs, we permanently labeled the pancreatic epithelium with a GFP reporter and traced their cell descendants by GFP expression. Despite robust GFP labeling of the epithelium, GFP expression was rarely identified in CAFs, suggesting little contribution of epithelium to the CAF pool. To determine whether the bone marrow gives rise to CAFs, we transplanted donor bone marrow carrying a ubiquitously expressed GFP reporter allele to GFP-negative recipient mice. We found that only a small proportion of pancreatic CAFs were tagged with GFP, suggesting their bone marrow origin. Lastly, to determine whether pancreatic TRFs give rise to CAFs, we used an inducible *CreER-LoxP* system to allow for permanent Tomato labeling in TRFs progenitors, the splanchnic mesenchyme, during mid-gestation. Lineage tracing in PDAC showed that the vast majority of CAFs were labeled with Tomato expression, suggesting their splanchnic origin. Furthermore, certain splanchnic gene expression signatures were persistent in subsets of CAFs in both the PDAC mouse model and human patient samples.

In summary, we found that bone marrow contributes to a small proportion of CAFs in PDAC, and the pancreatic epithelium contributes even less. Meanwhile, pancreatic TRFs are derived from the splanchnic mesenchyme during fetal development and they expand to contribute to the vast majority of CAFs in PDAC. Moreover, the persistence of splanchnic signature defines subtypes of CAFs. This study provides approaches to robustly target CAFs *in vivo* and novel insights into CAF heterogeneity in PDAC.

PR-014 Hedgehog represses angiogenesis in PDAC through a paracrine cascade mediated by Wif1. Marie C. Hasselluhn[1], Amanda R. Decker[1], Alvaro Curiel Garcia[1], Carlo Maurer[2], Dafydd Thomas[3], Kenneth P. Olive[1]. [1]Columbia University Irving Medical Center, New York, NY, [2]Technische Universität München, Munich, Germany, [3]PMV Pharmaceuticals, Inc., Cranbury, NJ.

Pancreatic Ductal Adenocarcinoma (PDAC) is characterized by an extremely heterogeneous tumor microenvironment (TME) paired with a sparse and compromised vascular network. These features of the TME shape drug responses and contributes to PDAC's distinctive chemotherapeutic resistance. Prior work demonstrated that inhibition of the Hedgehog (Hh) signaling using Smoothened (Smo) inhibitors relieved some of the local angiogenic suppression. However, the precise mechanisms through which the Hh pathway regulates angiogenesis has remained elusive. Subsequent studies evaluating different timepoints yielded conflicting data on the role of Hedgehog signaling in angiogenesis. In order to replicate the heterogeneity and spatial patterning of cells in pancreatic tumors, we developed a novel tumor explant system for short-term cultures of both murine and human PDAC. Briefly, we cultured intact 300 μm slices of PDAC tissues in a modified gelatin platform system with a novel culture medium informed by the metabolic composition of PDAC tumor interstitial fluid. This system replicates some of the nutrient/waste gradient of PDAC and enables tumors to be culture up to one week with good representation of multiple cell types. Studies in explants were complemented by work in the KPC genetically engineered model of PDAC. Comparison of short versus long-term Smo inhibition in KPC mouse pancreatic tumors revealed a robust increase in vessel density in short-term treatment, which is lost at later timepoints. Studies in murine and human PDAC explants employing different Smo inhibitors demonstrated increased endothelial tip cell formation upon two days of treatment, resulting in increased vessel density after four days. Computational analyses of human PDAC single cell RNAseq data identified candidate cellular subsets potentially involved in the signaling cascade. RNAseq of pre- and on-treatment tumor samples from KPC mice identified Wif1 as a candidate Hh target gene that effected these programs. Mechanistic studies in tumor explants reveal a complex, multi-step mechanism involving multiple cell types that led to suppression of angiogenesis in PDAC. These studies demonstrate how the tumor explant platform can elucidate complex intercellular signaling cascades and provide a candidate mechanism for the Hh-mediated suppression of angiogenesis in PDAC.

PR-015 Cancer-associated fibroblasts sustain critical dependency of pancreatic cancer cells on exogenous lipids. Charline Ogier[1], Alena Klochkova[2], Linara Gabitova[1], Battuya Bayarmagnai[3], Diana Restifo[1], Aizhan Surumbayeva[1], Janusz Franco-Barraza[1], Ralph Francescone[1], Debora B. Barbosa Vendramini-Costa[1], Jaye Gardiner[1], Emmanuelle Nicolas[4], Elizabeth A. Handorf[5], Kathy Q. Cai[6], Edna Cukierman[1], Igor Astsaturov[1]. [1]The Marvin & Concetta Greenberg Pancreatic Cancer Institute, Fox Chase Cancer Center, Philadelphia, PA,

[2]Temple University Lewis Katz School of Medicine, Philadelphia, PA, [3]Department of Bioengineering, Temple University, Philadelphia, PA, [4]Genomic Facility, Fox Chase Cancer Center, Philadelphia, PA, [5]Biostatistic Facility, Fox Chase Cancer Center, Philadelphia, PA, [6]Histopathology Facility, Fox Chase Cancer Center, Philadelphia, PA.

Pancreatic ductal adenocarcinoma (PDAC) cells derive their resistance to therapy and aggressive clinical course from the symbiotic signaling and metabolic interactions with cancer-associated fibroblasts (CAFs). CAFs have been shown to provide to metabolically "parasitic" PDAC cells with water-soluble metabolites such as glucose and amino acids, or bulk protein via micropinocytosis. To meet the elevated demands for cellular membrane lipids, cancer cells rely on uptake of the exogenous lipids. However, the mechanism by which PDAC cells obtain water-insoluble essential lipids, such as cholesterol, remains poorly understood. Here, we define CAFs as a main source of lipids for PDAC cells by direct "feeding" of the CAF plasma membrane (PM) to cancer cells via heterotypic cellular contacts, a phenomenon known as trogocytosis. To gain insights into the mechanisms of regulation of CAF trogocytosis, we engineered cholesterol-auxotrophic human and mouse PDAC cells. In the absence of exogenously provided cholesterol, these cancer cells undergo apoptosis, which is completely rescued in CAF co-cultures. Using CRISPRi-mediated depletion in CAFs of select genes involved in cholesterol trafficking, membrane fusion and membrane protrusions, we found that CAFs deficient in CD81, TMEM16F, or ARF6 exhibited markedly reduced ability to support the viability of cholesterol-auxotrophic PDAC cells in lipid-poor media. As a promising therapy target, TMEM16F is a Ca^{2+}-regulated scramblase increasing phosphatidylserine (PtdSer) on the outer leaflet of the PM. TMEM16F protein is highly expressed in human PDAC CAFs compared to fibroblasts isolated from the adjacent non-malignant pancreatic tissues. The TMEM16F-null CAFs are unable to sustain of cholesterol-auxotrophic PDAC cells in lipid-poor co-cultures. Our overall model is that, to initiate trogocytosis, PDAC cells release paracrine "feed me" signals activating Ca^{2+}-dependent phospholipid scramblase TMEM16F. As the result, increased outward expression of PtdSer on CAF PM is a hallmark "eat me" signal that is recognized by the trogocytic PDAC cells. We conclude that trogocytosis is the critical metabolic dependency in PDAC, and nominated TMEM16F as a plausible PDAC therapy target. Re-purposing of clinically available TMEM16 inhibitors will make a tangible impact on treatment of PDAC patients in the near term.

PR-016 Extrinsic KRAS signaling shapes the pancreatic microenvironment through fibroblast reprogramming. Ashley Velez-Delgado, Katelyn L. Donahue, Kristee L. Brown, Wenting Du, Valerie Irizarry-Negron, Rosa E. Menjivar, Emily L. Lasse-Opsahl, Nina G. Steele, Stephanie The, Jenny Lazarus, Veerin R. Sirihorachai, Filip Bednar, Timothy L. Frankel, Yaqing Zhang, Marina Pasca di Magliano. University of Michigan, Ann Arbor, MI.

Pancreatic Ductal Adenocarcinoma (PDA) has an exceedingly poor prognosis with only 10% 5-year survival rate. Kras mutations are found in over 90% of cases of pancreatic cancer and drive the formation of pancreatic intraepithelial neoplasia (PanIN), precursor lesions to PDA. Both PanIN and PDA are characterized by dense stroma, containing fibroblasts and immune cells. The infiltrating immune cells have a suppressive phenotype and prevent anti-tumor immunity by cytotoxic T cells. The mechanisms underlying the immunosuppression in pancreatic cancer are only partially understood. Our goal was to unravel the non-cell autonomous role of oncogenic Kras (Kras*) expressing epithelial cells in driving the formation of a complex, tumor promoting

microenvironment during the onset of pancreatic cancer. Our laboratory has described a mouse model (iKras*) of inducible and reversible expression of Kras* in the pancreas. Taking advantage of the reversibility of Kras* expression in this model, we conducted a thorough characterization of the immune infiltration and function upon modulation of Kras* at different stages of pancreatic carcinogenesis. iKras* mice and wild type littermates were enrolled in experiments at the age of 8-12 weeks. Kras* expression was activated, then we induced acute pancreatitis to promote the formation of pre-neoplastic lesions. After 3 weeks, a time when widespread low-grade lesions and fibrosis are observed, mice were either harvested or Kras* expression was inactivated and 3 days or 1 week later the pancreas was harvested. We performed flow cytometry, immunohistochemistry and cytometry time of flight (CyTOF) in the pancreas to analyze myeloid cell populations, as well as functional markers (Arg1, iNOS, IFN). Myeloid cells and T cells infiltrated the pancreas in presence of active Kras*. Inactivation of Kras* resulted in a relatively modest decrease in infiltrating myeloid cells. However, analysis of the functional marker Arg1, a putative immune suppressive molecule expressed in myeloid cells, indicated that its expression depends on Kras*-expressing cells. When we further studied the interactions among cell types using single cell RNA sequencing, we discovered that non-cell autonomous (extrinsic) oncogenic KRAS signaling reprograms pancreatic fibroblasts, activating an inflammatory gene expression program. As a result, fibroblasts become a hub of extracellular signaling, mediating the polarization and function of pro-tumorigenic myeloid cells while also preventing tissue repair.