Virtual Posters

Big Data

PO-001 ARNTL2 is a hypoxia-responsive master regulator of PDAC malignancy.
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Large-scale sequencing studies have identified a limited number of genetic drivers of PDAC malignancy, with only four genes found mutated a high penetrance across human pancreatic tumors. This suggests that the cell-autonomous ability to progress, dedifferentiate and metastasize is determined by non-genetic alterations to transcriptional regulation. Regulatory network analysis is a computational means of identifying changes in the activity of transcription factors on a genome-wide scale. Briefly, the approach calculates the activity of each regulatory protein (i.e. transcription factors, co-factors, and other proteins that regulate RNA transcript abundance) based on the expression of its positive and negative transcriptional targets (which are inferred *de novo* in a context-specific manner using the ARACNe algorithm). In this way, it is possible to identify the most hyper-activated and hyper-repressed regulatory proteins between groups of samples that represent different phenotypes – i.e. the "master regulators" (MRs) of those phenotypes. We applied regulatory network analysis to identify MRs of PDAC malignancy by comparing groups of samples representing several phenotypes. First, we performed laser capture microdissection and RNA sequencing (LCM-RNAseq) on 199 human PDAC samples, 26 low-grade PanIN, and 19 low-grade IPMN, isolating just the epithelial cells for transcriptional profiling. We then identified MRs of four different phenotypes among these samples: 1) precursor versus PDAC; 2) low-grade versus high-grade PDAC (based on histopathology); 3) low versus high Kras signaling; and long versus short overall survival. Integrating these analyses, the most highly activated regulatory protein in the genome across these four phenotypes was ARNTL2, an understudied member of the ARNT (HIF1β) superfamily. Notably, this protein was only modestly differentially expressed at the RNA level, indicating that its activation occurs primarily at a post-translational level. Computational analysis of the function of ARNTL2 indicated an enrichment of pathways involved in hypoxia responsiveness. Experimental analysis found that ARNTL2 had little impact in proliferation or survival under normoxic conditions, but drove increased invasiveness under hypoxic conditions. The functional associations of ARNTL2 in cultured cells expanded substantially under hypoxic conditions. Together, these findings suggest that ARNTL2 may serve as a Kras-associated, hypoxia-responsive driver of PDAC malignancy.

PO-002 Initial retrospective analysis of mechanisms of FOLFIRINOX resistance using clinical and molecular data from the Know Your Tumor (KYT) pancreatic ductal adenocarcinoma (PDAC) cohort.
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Introduction: The Know Your Tumor (KYT) pancreas cancer program enables molecular analysis of both tumor DNA and mRNA to characterize tumor phenotypes and guide potential treatment options. The primary objective of the current study was to develop and apply criteria to establish treatment response from real-world clinical data and to characterize the matching molecular and immunologic features of the pancreatic ductal adenocarcinoma (PDAC) tumor biopsies to identify tumor phenotypes that associate with treatment response. **Experimental Procedures:** Formalin-fixed, paraffin-embedded tumor samples and related clinical data were collected from n=240 patients diagnosed with PDAC and treated at medical centers in the United States. Samples underwent retrospective mutation analysis using the Tempus xT targeted panel of 648 genes and whole transcriptome RNA-sequencing also from Tempus. To identify molecular profiles associated with tumor treatment response, the duration of first therapy and CA19-9 levels taken after the start of first therapy were analyzed to establish consensus responder and non-responder criteria. Mutation associations using Fisher exact tests and differential gene expression and immune signature analysis using Wilcoxon tests were performed on all FOLFIRINOX-treated subjects that meet consensus responder (n = 12) or non-responder (n = 10) definitions to identify molecular underpinnings of FOLFIRINOX resistance. Gene expression analysis initially focused on tumor intrinsic genes (TIGs) because the expression of these genes may specifically reflect PDAC tumor biology. Differentially expressed TIGs with an unadjusted p-value < 0.05 were submitted for gene set enrichment analysis (GSEA) and manual investigation. **Results:** No association between DNA mutations and response to FOLFIRINOX was observed in this small dataset. In contrast, results from RNA-based gene expression analysis suggested that differences in tumor biology may contribute to response. GSEA and manual interrogation of differentially expressed TIGs revealed non-responder tumors may have high expression of apoptosis-related genes and genes associated with energetics. GSEA of all differentially expressed genes (unadjusted p-value < 0.05) refined this interpretation and corroborated initial findings by suggesting non-responders may have high expression of hypoxia, glycolysis- and apoptosis-related genes and low expression of *SMAD4* and its associated target genes. **Summary and Conclusions:** These results support the testable hypothesis that FOLFIRINOX non-responders evade apoptosis through hypoxia adaptations and autophagic flux. These hypotheses may warrant further investigation with preclinical models such as cell culture, PDX or organoid models. Finally, the analysis plan provides a roadmap for using the KYT cohort and other real-world datasets to generate hypotheses about molecular mechanisms of treatment response.

PO-003 Predictors for 30-day readmission in patients with pancreatic cancer who had DNR code status. Jasmeet Kaur[1], Tanveer Mir[2], Paramveer Singh[3], Judie Goodman[1]. [1]Saint Joseph Mercy Oakland Hospital, Pontiac, MI, [2]Wayne State University, Detroit, MI, [3]Karmanos Cancer Center, Detroit, MI.

Background: Pancreatic cancer is a lethal malignancy, and most patients present with advanced disease. There is little known about the 30-day readmission rate in patients with Do-not resuscitate (DNR) code status in pancreatic cancer. The study aims to look for predictors for 30-day readmission in pancreatic cancer patients with DNR code status. **Methods:** This is a retrospective study of a nationally representative cohort of hospitalized admissions admitted

from January 1, 2016, to December 31, 2018; 240,107 index pancreatic cancer hospitalizations were recorded. The database was obtained from the Agency for Healthcare Research and Quality's (AHRQ) Healthcare Cost and Utilization Project (HCUP) national readmission (NRD) dataset files between 2016 – 2018. We examined predictors of death and 30-day readmission among patients with pancreatic cancer who had DNR code status. We evaluated readmission in pancreatic cancer with DNR code status in multivariable Cox hazard regression models. **Results:** There were 240,107 index hospitalizations with pancreatic cancer (PAC) for the years 2016-2018. There were 51,451 (21.4%) PAC patients who had DNR code status during the index hospitalization. Patients with DNR status had a mean age of 68. The PAC patients with DNR status had significantly higher numbers of inpatient mortality (22% (DNR status) vs 3 % (full code) (OR 4.24 (95% CI 3.9-4.6; P <0.001), higher rate of cardiac arrhythmia (26% vs. 19%; p<0.001). The adjusted odd's ratio (Table 1.) to look for significant readmission predictors for DNR status in PAC included chronic heart failure (OR 1.24, p <0.001), renal failure (OR 1.27, p<.001), and liver disease (OR 2.13, p <0.001). However, patients with diabetes and obesity were found to have a negative association with readmission. Most patients were treated in urban teaching hospitals, and Medicare was the primary payor in 70.4%. **Conclusion:** In this large nationwide study, we observed higher inpatient mortality and readmission rates in pancreatic cancer who have DNR code status utilizing hospital resources and healthcare costs. This suggests that patients with advanced pancreas cancer who adopt DNR status be offered early hospice care to avoid inpatient mortality. There is a need to look for data based on racial and ethnic differences.

PO-004 Basal-like, Classical A, and Classical B subtypes of pancreatic cancer show distinct immuno-suppressive molecular profiles. Emily L. LaPlante, Dongliang Liu, Aleksandar Milosavljevic, Qizhi Yao. Baylor College of Medicine, Houston, TX.

With recent advances in distinguishing Pancreatic Ductal Adenocarcinoma (PDAC) subtypes based on transcriptional profiles, it is becoming clear that different subtypes have distinct therapeutic responses– e.g. Basal-like tumors tend to show less response to chemotherapy than Classical tumors. Here, we asked if the Basal-like, Classical A, and Classical B PDAC subtypes show distinct immuno-suppressive molecular profiles of relevance for targeted immunotherapy of PDAC. Toward this goal, we leveraged two large, public sequencing cohorts – TCGA and ICGC. To accommodate the large variation in cell type composition among the tumors across these cohorts, we applied the novel expression deconvolution (XDec) method to computationally deconvolute the bulk RNA-seq profiles. The method identified cell-type composition of each tumor and identified distinct Basal-like, Classical A, and Classical B subtypes, thus allowing classification of each tumor based on the state of its constituent cancer cell fraction. We further extended the method to also infer cell-type specific gene expression for each tumor sample and to identify correlation of cell-type specific gene expression levels across cohorts. Using the extended method, we determined that Basal-like cancer cells are *CD274* high, Classical B cancer cell *CD274* expression correlated strongly with *MSLN* and *RELA* (a recently proposed mechanism controlling PD-L1), and Classical A cancer cells had low *CD274* expression. Because heterotypic interactions may also contribute toward an immuno-suppressive microenvironment, we next correlated expression levels between cancer and stromal cell types. Cancer cell *MSLN* expression correlated strongly with stromal *CD274* expression which also correlated with stromal *IFNGR1* and IFNγ. Using co-culture experiments we validated the

causative relationship between cancer cell *MSLN* and stromal *INFGR1* and *CD274*. Taken together, our results suggest that PDAC subtypes have distinct therapeutic vulnerabilities, with Basal-like and *MSLN*-high Classical B patients more likely to respond to PD-L1 targeting therapies. Moreover, we provided a methodological advance by extending the XDec method and by making it web-accessible, thus allowing the community to derive hypotheses testable in cell line models by performing correlations of cell-type specific gene expression levels deconvoluted from bulk RNA-seq profiles of PDAC patient tumors.

PO-005 Proteome profiling of pancreatic ductal adenocarcinoma (PDAC) primary tumors in Caucasian, African American and Latinx patients. Henry C. H. Law[1], Andrea N. Riner[2], Jose G. Trevino[3], Nicholas T. Woods[1]. [1]University of Nebraska Medical Center, Omaha, NE, [2]University of Florida, Gainesville, FL, [3]Virginia Commonwealth University, Richmond, VA.

The clinical management of pancreatic ductal adenocarcinoma (PDAC) faces difficult challenges due to its aggressive metastatic potential, complex microenvironment, and lack of targeted therapies. Health disparities also exacerbate these challenges. For instance, African and African Americans have higher incidence rates and worse clinical outcomes than Whites even when socioeconomic and tumor stages are controlled. To advance the understanding of the biological differences across the racial groups, the PDAC primary tumors collected from 30 Caucasian, 12 African American (AA) and 3 Latinx patients were analyzed by quantitative proteomics. In collaboration with the IDeA National Resource for Quantitative Proteomics, 5820 proteins were identified and quantified using data-independent acquisition (DIA) in the tumor proteome. Comparing the Latinx and the Caucasian tumor proteome, 120 and 95 proteins were found up- and down-regulated in the Latinx proteome. Proteins involved in the fatty acid metabolism, urea cycle, bile acid and bile salt metabolism were found enriched among the upregulated proteins. 108 and 75 proteins were found up- and down-regulated in African American tumor proteome over the Caucasians. The 108 upregulated proteins were submitted for Reactome Pathway Analysis. Pathways such as the complement cascade, extracellular matrix organization and ECM proteoglycans were found enriched. Haptoglobin-related protein (HPR) was one of the 108 upregulated proteins in the AA tumor proteome, which is also observed at the transcript level in the TCGA data. The HPR is known for its trypanolytic function and gene amplifications are observed in those of African descent. HPR works with haptoglobin (HP) to clear the free hemoglobin in blood to prevent oxidative damage. We believe that the proteins overexpressed, and the biological processes activated are contributing to the PDAC disparities observed in the African descendants. Therefore, the characterization of the PDAC proteome is a valuable method to delineate the underlying molecular signatures that may contribute to the health disparities.

Diagnostics, Early Detection, and Imaging

PO-006 CircRTN4 promotes pancreatic cancer progression through a novel circRNA-miRNA-lncRNA pathway and stabilizing epithelial-mesenchymal transition protein. Chi Hin Wong[1], Ut Kei Lou[1], Frederic Khe-Cheong Fung[1], Joanna H. M. Tong[2], Ka-Fai To[2], Stephen Lam Chan[3], Yangchao Chen[4]. [1]School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China-Hong Kong, [2]Department of Anatomical and Cellular Pathology, Prince of Wales Hospital, The Chinese University of Hong

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Background & Aims: Circular RNAs (circRNAs) play important roles in many biological processes. However, the detailed mechanism underlying the critical roles of circRNAs in cancer remains largely unexplored. We aim to explore the molecular mechanisms of circRTN4 with critical roles in pancreatic ductal adenocarcinoma (PDAC). **Methods:** CircRTN4 expression level was examined in PDAC primary tumors. The oncogenic roles of circRTN4 in PDAC tumor growth and metastasis were studied in mouse tumor models. Bioinformatics analysis, luciferase assay and miRNA pulldown assay were performed to study the novel circRTN4-miRNA-lncRNA pathway. To identify circRTN4-interacting proteins, we performed circRNA-pulldown and mass spectrometry in PDAC cells. Protein stability assay and 3-Dimensional structure modeling were performed to reveal the role of circRTN4 in stabilizing RAB11FIP1. **Results:** circRTN4 was significantly upregulated in primary tumors from PDAC patients. *In vitro* and *in vivo* functional studies revealed that circRTN4 promoted PDAC tumor growth and liver metastasis. Mechanistically, circRTN4 interacted with tumor suppressor miR-497-5p in PDAC cells. CircRTN4 knockdown upregulated miR-497-5p to inhibit the oncogenic lncRNA HOTTIP expression. Furthermore, we identified critical circRTN4-intercting proteins by circRNA-pulldown in PDAC cells. CircRTN4 interacted with important epithelial-mesenchymal transition (EMT)- driver RAB11FIP1 to block its ubiquitination site. We found that circRTN4 knockdown promoted the degradation of RAB11FIP1 by increasing its ubiquitination. Also, circRTN4 knockdown inhibited the expression of RAB11FIP1-regulating EMT-markers Slug, Snai1, Twist, Zeb1 and N-cadherin in PDAC. **Conclusion:** The upregulated circRTN4 promotes tumor growth and liver metastasis in PDAC through the novel circRTN4-miR-497-5p-HOTTIP pathway. Also, circRTN4 stabilizes RAB11FIP1 to contribute EMT.

PO-007 Plasma-based detection of pancreatic cancer: A multiomics approach. Teng-Kuei Hsu[1], Tzu-Yu Liu[1], Billie Gould[1], Christine Decapite[2], Amer Zureikat[3], Alessandro Paniccia[3], Eric Ariazi[1], Marvin Bertin[1], Richard Bourgon[1], Kaitlyn Coil[1], Hayley Donnella[1], Adam Drake[1], Julie M. Granka[1], Preet Kaur[1], Maggie C. Louie[1], Shivani Mahajan[1], Amit Pasupathy[1], Ofer Shapira[1], Peter Ulz[1], Chun Yang[1], C. Jimmy Lin[1], Randall Brand[2]. [1]Freenome Holdings Inc., South San Francisco, CA, [2]Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA, [3]Department of Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA.

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers, with an overall five-year survival rate of 11%. Potential curative resection is possible if the tumor is detected at an early stage, with a five-year survival rate of 42%. The only current FDA-cleared biomarker for PDAC is the carbohydrate antigen 19-9 (CA19-9), which is intended for monitoring response to therapy but not for early detection. CA19-9 blood tests have varying sensitivity to detect PDAC and are prone to false positives in the presence of other underlying pancreatic conditions and to false negatives in subpopulations unable to express CA19-9. The goal of our pilot study was to determine if a multiomics approach using cell-free DNA and CA19-9 would be better than CA19-9 alone in detecting PDAC. In this retrospective study of 75 participants, we performed

targeted methylation profiling of circulating cell-free DNA and quantitation of plasma CA19-9 abundance. Participants with PDAC (n=39) were 51% male with a mean age of 74.9 years, and consisted of stage II (n=9), stage III (n=11) and stage IV (n=19) pancreatic cancer. Controls (n=36) were 33% male with a mean age of 74.2 years, and included both healthy control/normal pancreas (n=17) and various benign abnormalities of the pancreas or biliary system (n=19). We developed a novel machine learning model that combines CA19-9 and methylation signals to build a joint multiomics prediction. We compared the joint predictions to those based on methylation or CA19-9 alone. Five resamplings of three-fold cross-validation were performed, and sensitivity was calculated for decision thresholds that achieved the desired test set specificity. Across all stages, the multiomics approach achieved a sensitivity of 93% at a specificity of 96%, which was greater than methylation or CA19-9 alone. At 96% specificity, methylation alone achieved a sensitivity of 74% while CA19-9 alone achieved a sensitivity of 87%. In stages II, III and IV, the multiomics approach achieved a sensitivity of 82%, 89%, and 100%, respectively and was also more sensitive than either methylation or CA19-9 alone. These proof-of-concept data demonstrate the promise of using a multiomics approach to develop a highly sensitive and specific test for the early detection of pancreatic cancer. Additional studies are underway, focusing on early-stage disease (stage I/II), to validate these results.

PO-008 Diagnostic accuracy of blood-based multi-omic biomarkers for pancreatic adenocarcinoma: A systematic review and meta-analysis. Laura E. Kane[1], Gregory S. Mellotte[2], Eimear Mylod[1], Rebecca O'Brien[1], Fiona O'Connell[1], Khanh Nguyen[3], Croí E. Buckley[1], Jennifer Arlow[3], David Mockler[1], Aidan D. Meade[3], Barbara M. Ryan[2], Stephen G. Maher[1]. [1]Trinity College Dublin, Dublin, Ireland, [2]Tallaght University Hospital, Dublin, Ireland, [3]Technological University Dublin, Dublin, Ireland.

Background: Pancreatic ductal adenocarcinoma (PDAC) is the most lethal form of pancreatic cancer, being responsible for ~90% of all pancreatic cancers and having a 5-year survival rate of ~8.5%. The current clinical gold-standard for diagnosis of PDAC is the blood-based biomarker CA19-9. However, many studies have highlighted the limitations of CA19-9, specifically its relatively low sensitivity and specificity, and its inaccuracy in patients with certain underlying conditions. As such, there is an unmet need for robust diagnostic biomarkers for PDAC. Here, the diagnostic accuracy of all blood-based biomarkers examined in PDAC, reporting specifically on CA19-9, multi-marker panels containing CA19-9, novel single markers, and novel multi-marker panels for the diagnosis of PDAC. **Methods:** A systematic review of blood-based biomarkers for the diagnosis of PDAC was conducted in accordance with PRISMA standards. Individual search strategies using medical subject headings (MeSH) and 'text words' were developed for three academic databases: Medline, EMBASE and Web of Science. The 5,885 studies identified were subjected to two rounds of screening by two independent reviewers, with 250 studies being included in the meta-analysis. Data were extracted and assessed for bias using the QUADAS-2 Risk of Bias tool. Data were separated into two subgroups: those including CA19-9, and those without CA19-9 (novel). Patient cohorts examined were classified as "PDAC vs healthy", "PDAC vs benign" and "PDAC vs mixed". A multivariate three-level meta-analysis with subgroup moderators was run in R (v1.3.959) on all CA19-9 containing biomarker studies and subsequently on all novel biomarker studies, using reported AUC values as effect size. **Results:** Based on the three-level meta-analytic model, the pooled AUC value for CA19-9 alone (AUC=0.8473, 95% CI: 0.82-0.87) was significantly lower compared to the multi-marker panels

containing CA19-9 (AUC=0.91, 95% CI:0.90-0.93) (p<0.0001). The estimated between-study variance in the model was $I^2_{\text{Level 3}}$= 63.55%, and the within-study variance was $I^2_{\text{Level 2}}$=36.45%. For the novel markers, the pooled AUC for single markers (AUC=0.79, 95% CI:0.75-0.83) was also significantly lower compared to novel multi-marker panels (AUC=0.87, 95% CI:0.85-0.89) (p<0.0001). Marker robustness was also influenced by the patient cohort examined, with CA19-9 markers performing best in all cohorts compared to novel markers; PDAC vs healthy (AUC=0.91, 95% CI:0.88-0.94), PDAC vs benign (AUC=0.85, 95% CI:0.84-0.87), and PDAC vs mixed (AUC=0.87, 95% CI:0.82-0.91) (p<0.0001). **Conclusion:** Overall, multi-marker panels show higher pooled AUC values than single markers, for both CA19-9 and novel datasets. Multi-marker panels containing CA19-9 demonstrate the most promising pooled AUC value, with CA19-9 alone performing inferiorly to novel multi-marker panels. These results indicate that CA19-9 may be best used as an addition to a panel of markers rather than alone, and that multi-marker panels ultimately generate the most robust results in a diagnostic capacity.

PO-009 Multi-omic profiling of patient pancreatic cyst fluid for the identification of a novel biomarker panel of patient cancer risk. Laura E. Kane[1], Gregory S. Mellotte[2], Simone Marcone[1], Barbara M. Ryan[2], Stephen G. Maher[1]. [1]Trinity College Dublin, Dublin, Ireland, [2]Tallaght University Hospital, Dublin, Ireland.

Background: Pancreatic cancer was responsible for almost 500,000 deaths globally in 2020 according to GLOBOCAN 2020. Pancreatic cystic lesions (PCLs) are fluid-filled protrusions either on or inside the pancreas. PCLs can either be benign or pre-malignant, however, current guidelines based on clinical features are limited in their ability to accurately stratify patients based on cancer risk. Multi-omic profiling of the pancreatic cyst fluid (PCF) could aid in the identification of a novel biomarker panel of patient cancer risk. **Methods:** PCF was collected from 40 patients by EUS-FNA. Patients were stratified using the 2018 European evidence-based guidelines into low-risk (n=15), high risk (n=15) and no-risk/pseudocyst (n=10). PCF was sonicated and subsequently processed using a single-pot solid-phase-enhanced sample preparation (SP3) protocol with Sera-Mag SpeedBead carboxylate-modified beads prior to LC-MS. Samples were run on a Thermo Scientific Q Exactive mass spectrometer coupled to a Dionex Ultimate 3000 (RSLCnano) chromatography system. MS-generated proteomic data were analysed in Perseus (v1.6.13). HTG microRNA whole transcriptome sequencing was run on whole PCF. Transcriptomic data were analysed using HTG EdgeSeq Reveal (v3.1). **Results:** MS-analysis revealed 1,266 proteins present across all PCF samples. Proteins were filtered based on potential contaminants and valid values. Only proteins expressed in a minimum of six PCF samples were included in the analysis. After data clean-up, 465 proteins were examined for differential expression. A total of eight proteins were upregulated in high-risk PCF compared to low-risk (p<0.05, FDR=0.05, s0=0.1). Among them, seven have been shown to be upregulated in pancreatic cancer. Conversely, one protein which is reported to be downregulated in pancreatic cancer, was significantly upregulated in the high-risk patient cohort. A total of 2,096 miRNAs were identified across all PCF samples. MiRNAs were filtered based on fold-change > ±2 between the groups, with 202 miRNAs meeting this criteria. Forty-six miRNAs were significantly upregulated in high-risk PCF compared to low-risk PCF (adj-p<0.05, FDR=0.05, s0=0.1). Five of these miRNAs are known to be upregulated in pancreatic ductal adenocarcinoma (PDAC) tissues. Furthermore, three of the miRNAs are upregulated in the circulation of PDAC patients. Importantly, seven miRNAs identified as being upregulated in

high-risk PCF have been shown to be downregulated in PDAC tissues. Differentially expressed proteins and miRNAs are currently being utilised to create an integrated, multi-omic predictive algorithm for patient risk. **Conclusion:** Multi-omic profiling of pancreatic cyst fluid provides an abundance of potential biomarkers that could be utilised for the stratification of patients into high-and low-risk groups for malignancy. Integration of multi-omic data has the potential to provide more robust biomarker panels of patient risk. Validation of biomarkers in independent patient cohorts will be key to the development of novel clinical biomarkers.

PO-010 Detection of early tissue changes on historical CT scans in the regions of the pancreas gland that subsequently develop adenocarcinoma using quantitative textural analysis and fat fraction analysis. Ronald L. Korn[1], Daniel D. Von Hoff[2], Andre Burkett[1], Dominic Zygadlo[1], Taylor Brodie[3], Kathleen Panak[1], Sweta Rajan[1], Derek Cridebring[2], Michael J. Demeure[3]. [1]Imaging Endpoints, Scottsdale, AZ, [2]Translational Genomics Research Institute, Phoenix, AZ, [3]Hoag Hospital, Newport Beach, CA.

Early detection of Adenocarcinoma of the Pancreas (ACP) is critical to improving outcomes. Since the development of ACP is thought to begin a couple decades prior to clinical presentation, the possibility exists that evolving changes in the pancreas gland (PG) may already be present on historical standard of care (h-SOC) CT scans obtained years earlier in patients who present for other indications. Advanced image analysis using quantitative texture analysis (QTA) techniques can detect subtle changes in tissue/tumors composition (including fat) and may be useful in tumor detection, diagnosis and response assessments. We hypothesized that changes in tissue texture are detectable on h-SOC in patients who subsequently develop ACP and, if identified, would contribute significantly towards the development of tools to aid in identifying tissue at risk for ACP An IRB-exempt, retrospective, single institution study of 27 matched h-SOC and ACP diagnostic CTs from a single institution was performed. Subjects who had ACP and h-SOC CTs between 3-15 years prior were included. Deidentified scans were transferred to the imaging core lab for QTA and fat fraction (FF) analysis. The pancreas gland (PG) was divided into 7 regions (uncinate, head, neck-genu, body [prox,mid,dis] and tail) for volumes-of interest (VOIs) placement on the h-SOC portal venous phase axial images. A single radiologist verified VOI placement, confirmed the lack of ACP on h-SOC images and was blinded to the location of subsequent ACP development. First order QTA histogram frequency curves derivatives (mean, SD, skewness, kurtosis, mean positive Pixel (MPP), at each cluster [SSF] setting from 0-6mm) were recorded along with FF. Inferential statistical analysis was performed to identify QTA/FF differences between PG regions that subsequently developed ACP from those that did not ($p < 0.05$ significance). A total of 22/27 subjects (81%) had suitable portal venous phase CTs for QTA while evaluable FF data was available for all subjects. ACP developed in PG head in 45% of subjects. The average time from h-SOC to diagnostic ACP scan was 7.6 years (range, 3.9 years-13.9 years). QTA results showed a difference in tissue texture ($QTA_{skewness} > -0.480$) in PG regions that subsequently developed ACP compared to regions that did not (T-statistics= -2.148; AUC_{ROC}= 0.625 p=.038). There was also a > 3-fold increase in PG tail fat in subjects that developed tail ACP (t-statistics= -3.048; p = 0.002; AUC_{ROC}= 0.819 p=.023). LOGR models showed that PG regions that subsequently developed ACP had differences in QTA mean, skewness, kurtosis and total FF on h-SOC scans compared to normal PG tissue (LL ratio-p=.004, pseudo R2=.104). Tissue texture and fat composition differences in regions of the PG may be present on h-SOC CT scans several years prior to clinical

manifestation of ACP. If validated, tissue at risk could be identified well in advance of actual ACP development allowing for new opportunities for PAC interception. The investigators acknowledge the Marley Foundation for their generous support.

PO-011 The spectrum of pathogenic germline variants in pancreatic cancer patients with multiple primary tumors. Valentyna Kryklyva[1], Lodewijk A. A. Brosens[2], Marjolijn J. L. Ligtenberg[1], Iris D. Nagtegaal[1]. [1]Radboud University Medical Center, Nijmegen, Netherlands, [2]University Medical Center Utrecht, Utrecht, Netherlands.

Introduction: Approximately 10% of pancreatic ductal adenocarcinomas (PDAC) are considered hereditary due to pathogenic germline variants in cancer predisposition genes. It is crucial to recognize hereditary PDAC to ensure surveillance of affected individuals and their relatives, and to identify the underlying molecular defects for application of targeted therapies. **Methods:** The study cohort includes 207 patients with PDAC and additional primary tumors in personal history selected from the Dutch Pathology Archive. Germline DNA was analyzed using two customized targeted next-generation sequencing panels including 26 cancer predisposition genes. Identified variants were checked for pathogenicity. **Results:** The study includes 207 patients diagnosed with PDAC in combination with two other primary tumors (n = 51), breast (n = 51), colorectal (n = 41), prostate (n = 18), ovarian (n = 8), gastric (n = 5), endometrial (n = 4) carcinomas, and melanoma (n = 29). Germline sequencing identified pathogenic or likely pathogenic variants (PV/LPV) in 43/207 (20.8%) of the patients. Per combination, PV/LPV were detected in PDAC patients with ovarian (4/8), gastric (2/5), melanoma (9/29), breast (12/51), prostate (3/18), colorectal (6/41), and two other primary (7/51) cancers. The most frequently affected genes were *ATM* (14/43), *BRCA2* (7/43; one patient with a concurrent *PMS2* LPV), *CDKN2A* (7/43; 5/7 with Dutch founder p16-*Leiden* PV), and *TP53* (3/43). Eight patients carried PV/LPV in *BRCA1* (2/43), *MSH6* (2/43), biallelic *NTHL1* (2/43), and *PALB2* (2/43) genes. Four patients each had a single PV/LPV in *BRIP1* (1/43), *CDH1* (1/43), *CHEK2* (1/43), and *RAD51C* (1/43). Importantly, more than a half of identified PV/LPV (28/43) occurred in double-strand DNA damage repair genes (14 *ATM*, 7 *BRCA2*, 2 *BRCA1*, 2 *PALB2*, 1 *BRIP1*, 1 *CHEK2*, and 1 *RAD51C*), potentially conferring a sensitivity to PARP inhibitors and platinum-based chemotherapies. Two patients (2/43) had *MSH6* PV, predisposing to Lynch syndrome, potentially responsive to immunotherapies. Overall, almost 70% of PV/LPV carriers and about 14.5% of all cases have potentially targetable germline defects. The median age at PDAC in PV/LPV carriers was only slightly younger (64 years, range: 36 – 82) than in non-carriers (66 years, range: 42 – 84), however, the difference was not significant. **Conclusions:** Up to 20% of patients with PDAC and multiple primary tumors have a hereditary predisposition to cancer onset; the majority harbor PV/LPV in double-strand DNA damage repair genes. Up to 15% of PDAC patients with multiple primaries and up to 70% of PV/LPV carriers have potentially targetable germline defects. Age-based preselection has limited utility to identify hereditary PDAC, however, the presence of specific other malignancies in personal history strongly indicates on underlying genetic predisposition, and should warrant further investigation and genetic screening.

PO-012 The concept of artificial intelligence against pancreatic cancer. Subash Kumar. DMI Lochbridge, Elkridge, MD.

Pancreatic cancer (PC) remains the fourth leading cause of cancer-related death in both men and women in the United States. Despite considerable research efforts, pancreatic cancer is associated with a dire prognosis and a 5-year survival rate of only 10%. Early symptoms of the disease are mostly nonspecific. The premise of improved survival through early detection is that more individuals will benefit from potentially curative treatment. Pancreatic ductal adenocarcinoma (PDAC) is on track to become the number 2 cancer killer in the United States within the next decade unless there is a major improvement in outcomes. Surgical resection remains the only reasonable hope for a cure from PDAC. The potential for early detection of asymptomatic pancreatic neoplasms in high-risk individuals using an endoscopic approach, but this approach is operator dependent and at the same time, these existing techniques are favored once patients reach the age of 75 years. Artificial intelligence (AI) methodology has emerged as a successful tool for risk stratification and identification in general health care. Machine learning refers to the study of algorithms that learn their behavior from data. To see why such algorithms are important, consider the following basic task, building a program to predict if an image contains a dog or a cat. Although it is exceedingly difficult for us to manually specify the exact rules to determine that a dog is a dog, it is comparatively straightforward to prepare a reference set of images and labels. This setting, where knowledge is more easily encoded in data rather than as a descriptive set of rules, is the focus of ML algorithms. One of the most promising areas of innovation in medical imaging in the past decade has been the application of deep learning. Deep learning has the potential to impact the entire medical imaging workflow from image acquisition, image registration, to interpretation. Traditional image processing is dominated by algorithms that are based on statistical models. These statistical model-based processing algorithms carry out inference based on a complete knowledge of the underlying statistical model relating the observations at hand and the desired information and do not require data to learn their mapping. In practice, accurate knowledge of the statistical model relating the observations and the desired information is typically unavailable. The past decade has witnessed a deep learning revolution. Deep learning methods have surpassed the state of the art for many problems in signal processing, imaging, and vision with unprecedented performance gains.

PO-013 Comparison of novel healthcare delivery models on the uptake of genetic education and testing in families with a history of pancreatic cancer: The GENetic Education, Risk Assessment and TEsting (GENERATE) study. Nicolette J. Rodriguez[1], Constance S. Furniss[2], Matthew B. Yurgelun[3], Chinedu Ukaegbu[4], Pamela E. Constantinou[5], Alison N. Schwartz[4], Jill Stopfer[4], Meghan Underhill-Blazey[6], Barbara Kenner[7], Scott Nelson[8], Sydney Okumura[9], Sherman Law[9], Alicia Y. Zhou[9], Tara B. Coffin[10], Hajime Uno[2], Allyson Ocean[11], Florencia McAllister[5], Andrew M. Lowy[12], Scott M. Lippman[12], Alison P. Klein[13], Lisa Madlensky[12], Gloria M. Petersen[14], Judy E. Garber[1], Michael G. Goggins[13], Anirban Maitra[5], Sapna Syngal[3]. [1]Dana-Farber Cancer Institute / Brigham and Women's Hospital / Harvard Medical School, Boston, MA, [2]Dana-Farber Cancer Institute / Harvard Medical School, Boston, MA, [3]Dana-Farber Cancer Institute / Brigham and Women's Hospital / Harvard Medical School, Boston, MA, [4]Dana-Farber Cancer Institute, Boston, MA, [5]Sheikh Ahmed Center for Pancreatic Cancer Research / University of Texas MD Anderson Cancer Center, Houston, TX, [6]University of Rochester, Rochester, NY, [7]Kenner Family Research Fund, New York, NY, [8]Pancreatic Cancer Action Network Volunteer, Manhattan Beach, CA, [9]Color Genomics, Burlingame, CA, [10]University of Washington, Seattle, WA, [11]Weill Cornell Medical Center, New York, NY, [12]Moores Cancer Center / UC San Diego, San Diego, CA, [13]Johns Hopkins University / Sol

Goldman Pancreatic Cancer Research Center, Baltimore, MD, [14]Mayo Clinic, Rochester, MN.

Background: Roughly 7–10% of patients with pancreatic ductal adenocarcinoma (PDAC) have a deleterious germline variant. Although identification of germline variants in family members has implications for cancer surveillance and can lead to early cancer detection and interception for PDAC, as well as other cancers, cascade genetic testing rates are low. The GENetic Education, Risk Assessment and TEsting (GENERATE) study evaluates novel methods of providing genetic education and testing for individuals at risk for hereditary PDAC. **Methods**: Eligible participants had: (1) a first- or second-degree relative with a diagnosis of PDAC and a known familial germline variant in *APC, ATM, BRCA1, BRCA2, CDKN2A, EPCAM, MLH1, MSH2, MSH6, PALB2, PMS2, STK11,* or *TP53* (Known Familial Mutation (KFM)), (2) or were first-degree relatives of PDAC patients (no KFM). Participants were recruited through six academic centers, patient advocacy organizations and online outreach. Enrollment occurred through the study website (www.GENERATEstudy.org). All study participation, including genetic testing via a at home saliva sample kit, was done remotely. Participants were cluster randomized at the family level into one of two arms. Arm 1 (Doxy.me plus Color Genomics) included remote genetic education and testing through a video-based telemedicine platform (Doxy.me) and physician-mediated testing through Color Genomics. Arm 2 included remote genetic education and testing through Color Genomics only. **Results**: Between 5/8/2019–6/01/2021, 423 families were randomized, comprising 595 participants. Recruitment occurred through patient invitation via healthcare providers (n=128, 21.5%), family members (n=271, 45.5%), friends, advocacy groups, and online outreach (n=223, 37.5%). Participants were referred from the six GENERATE academic centers (n=270, 45.4%) and other institutions (n=325, 54.6%). Study participants were 52.5 years on average, primarily identified as White (n=577, 97%) and from the Northeast (n=184, 30.9%), Midwest (n=154, 25.9%), South (n=158, 26.6%) and West (n=99, 16.6%). Participants were randomized into each arm (n=296 Doxy.me plus Color Genomics; n=299 Color Genomics only). To date, 527 (88.6%) participants have ordered genetic testing. The uptake of genetic testing was 253/296 (85.5%) in the Doxy.me plus Color Genomics arm and 274/299 (91.6%) in the Color Genomics only arm (p=0.049, generalized mixed-effects model). A total of 82 PDAC associated pathogenic variants were identified. The most frequently detected variants were *BRCA2* (n=32), *ATM* (n=25) and *PALB2* (n=6). Additionally, 13 non-PDAC associated pathogenic variants and 20 low penetrance variants were detected. **Conclusions**: Remote methods of genetic education and testing are successful alternatives to traditional cascade testing, with genetic testing rates nearly 90%. Participant follow up will assess if satisfaction with decision making, cancer-risk distress, knowledge gained, family communication, and uptake of surveillance were impacted by the mode of delivery of pre-test genetic education.

PO-014 VISTA: VIsual Semantic Tissue Analysis for pancreatic disease quantification in murine cohorts. Luke Ternes, Ge Huang, Christian Lanciault, Guillaume Thibault, Rachelle Riggers, Joe Gray, John Muschler, Young Hwan Chang. Oregon Health and Science University, Portland, OR.

Objective and quantifiable assessment of tissue pathology is necessary to study mechanistic disease progression; however, current quantification methods based on tissue staining have many drawbacks including cost, time, labor, batch effects, as well as uneven staining which can result

in misinterpretation and investigator bias. Here we present VISTA, an automated deep learning tool for semantic segmentation and quantification of histologic features from hematoxylin and eosin (H&E) stained pancreatic tissue sections. VISTA is trained to identify four key tissue types in developing murine PDAC samples: normal acinar, acinar-to-ductal metaplasia (ADM), dysplasia, and other normal tissue. Predicted segmentations were quantitatively evaluated against pathologist annotation with Dice Coefficients, achieving scores of 0.79, 0.70, 0.79 for normal acinar, ADM, and dysplasia, respectively. Predictions were evaluated against biological ground truth using the mean structural similarity index to immunostainings amylase and pan-keratin (0.925 and 0.920, respectively). The total area of feature prediction was also correlated to the area of immunostaining in whole tissue sections using spearman correlation (0.86, 0.97, and 0.92 for DAPI, amylase, and cytokeratins, respectively). Importantly, our tool is not only able to predict staining information, but it is able to distinguish between ADM and dysplasia, which are not reliably distinguished with common immunostaining methods, showing VISTA's potential to expand research beyond what is capable with current standards. As a use case example of VISTA, we quantified abundance of histologic features in murine cohorts with oncogenic Kras-driven disease. We observed stromal expansion, a reduction in normal acinar, and an increase in both ADM and dysplasia as the disease progresses, which matches known biology. Since VISTA is an automated algorithm, it can accelerate histological analysis and improve the consistency of quantification between laboratories and investigators. This work has been published in Nature Scientific Reports, and the code is available on github at https://github.com/GelatinFrogs/MicePan-Segmentation.

Early Phase Clinical Trials

PO-015 A phase Ib/II trial of high dose ascorbic acid (AA) + paclitaxel protein bound (PP) + cisplatin (C) + gemcitabine (G) in patients (pts) with previously untreated metastatic pancreatic cancer (MPC). Gayle S. Jameson[1], Erkut H. Borazanci[1], Daniel D. Von Hoff[2], Joshua D. Rabinowitz[3], Michael S. Gordon[1], Sarah D. LeGrand[1], Courtney Snyder[1], Karen Ansaldo[1], Denise J. Roe[4], Haiyong Han[2]. [1]HonorHealth, Scottsdale, AZ, [2]Translational Genomics Research Institute (TGen), Phoenix, AZ, [3]Princeton University, Princeton, NJ, [4]University of Arizona Cancer Center, Tucson, AZ.

Background: Increasing evidence suggests that high concentrations of AA (vitamin C) can decrease the growth of aggressive tumors, including Ras-mutant tumors. Based on the unmet need in MPC, its high frequency of Ras mutations, and prior work combining PP +C +G in pts with MPC (response rate of 70.5%, median survival 16.4 mos), we explored the addition of AA to that regimen. Pre and post treatment biopsies for biomarkers and digital spacial profiling are also being performed. **Methods:** This pilot trial utilized a 3 + 3 design for dose escalation of AA. Eligibility criteria included untreated stage IV MPC, ECOG 0-1, and measurable disease. Excluded were pts with a G6PD deficiency, renal oxalate stones, or need of capillary blood glucose monitoring as AA causes false low readings. The primary objective of the phase 1b was to determine the maximum tolerated dose (MTD) of AA in combination with PP +C +G in pts with MPC. Exploratory objectives included analysis of tumor texture on radiologic scans as an imaging biomarker for response; correlation between peak plasma levels of AA and response to treatment; potential biomarkers in the tumor including tumor immune cell infiltration, stromal activation, stem call enumeration; change in numbers of circulating tumor stem cells and

macrophage lineage changes. **Results:** As of 7/2021, the phase Ib enrollment in this study has been completed. Six pts were treated in the cohort of AA 25 gm/m2, 4 pts at AA 37.5 gm/m2, and 7 pts at 56.25 gm/m2. The majority of all reported AEs were grades 1-2. The most common AEs related to study treatment were: platelet count decrease (70.6%), diarrhea (70.6%), hypomagnesemia (52.9%), dysgeusia (47.1%), peripheral sensory neuropathy (47.1%), nausea (35.3%), fatigue (35.3%), neutrophil count decreased (35.3%), and hypokalemia (35.3%). Of the 17 pts enrolled, 15 were evaluable. Response by RECIST 1.1 is 11 PR (73.3%), 2 SD (13.3%), 2 PD (13.3%). Evaluation of median PFS and OS is ongoing. **Conclusions:** This study has a high response rate of 73%. Most pts in all AA dosing cohorts experienced a brisk response to therapy. AA did not appear to add toxicities compared to historical AE data of PP +C +G and was well tolerated in all dose cohorts. The MTD of AA was not reached in the 56.25 gm/m2 cohort. The protocol allowed for an additional dose escalation of AA to 75 gm/m2. However, all pts in the 56.25 gm/m2 cohort achieved our goal of peak plasma AA levels of > 20 mM so the study was closed to enrollment. Quality of life measures, tumor tissue and blood sample analyses are underway. Supported by SU2C, Cancer Research UK, Lustgarten Foundation, Destroy Pancreatic Cancer & the Young family.

Immunotherapy

PO-016 Directed evolution generates novel oncolytic H-1 parvoviruses with improved therapeutic efficacy in virus-resistant pancreatic cancer cells. Pierre Garcin[1], Monireh Kazemimanesh[1], Hubert Lulka[1], Nelson Dusetti[2], Guillaume Labrousse[1], Emilie Benuzzi[1], Louis Buscail[3], Pierre Cordelier[1]. [1]Cancer Research Center of Toulouse, INSERM, Toulouse, France, [2]Cancer Research Center of Marseilles, INSERM, Marseilles, France, [3]Cancer Research Center of Toulouse, INSERM and Toulouse University Hospital, Toulouse, France.

Despite considerable promise and emerging clinical success, several challenges impede the broader implementation of novel immunotherapies such as oncolytic virus(OV)-based gene therapy, including for patients with pancreatic cancer (PDAC). One of such challenge is inter-patient variability that may impact on OV selectivity and killing efficacy for tumor cells. For this study, we selected the rat parvovirus H-1 (H-1PV) that is nonpathogenic in humans and has a natural oncolytic activity in several cancer models. The safety and tolerability of H-1PV was recently demonstrated in early clinical trials for glioma and PDAC. However, we report here that H-1PV infection, oncolytic and pro-apoptotic activity are limited in PDAC cells, including patient-derived primary cells. To address this concern, we applied a directed evolution strategy to generate H-1PV variants with specific activity towards PDAC cells. Following selection using patient primary cells, we managed to isolate clonal, PDAC-adapted H-1PVs that induce PDAC cells lysis as compared to parental H-1PV while infection of normal pancreatic cells remained negligeable. Genome sequencing of the tumor adapted virus reveals mutations in promoting and viral capsid sequences. *In vivo*, the tumor-adapted H1PV demonstrates greater anti-tumor effect than parental H-1PV, following intravenous administration in an experimental model of orthotopic pancreatic tumors engrafted in immunodeficient mice. To our knowledge, we report here for the first time the production of highly selective and potent OV using directed evolution to override PDAC resistance to virotherapy. While the molecular mechanisms involved are still under investigation, this project is a first step towards precision medicine strategies based on OV.

PO-017 Application of oncolytic adenovirus to desmoplastic pancreatic cancer. Elora Hossain, Fumihiro Higashino. Hokkaido University, Sapporo, Japan.

Pancreatic ductal adenocarcinoma (PDAC) is one of the highly malignant tumors with the poorest prognosis worldwide. The five-year survival rate of pancreatic cancer patients is 5%. PDAC comprises a dense fibrotic stroma with an extracellular matrix produced by activated-human pancreatic stellate cells (HPSC), which helps tumor growth, infiltration and metastasis. It is the main reason for chemotherapy resistance since fibrotic stroma is impenetrable for drug delivery. AU-rich elements (ARE) are RNA elements commonly present in the 3'-UTR of certain mRNAs that encode many early response genes or growth-related genes such as proto-oncogenes. ARE enhances the rapid decay of mRNAs, and the fate of ARE-mRNA is controlled by ARE-binding proteins HuR. ARE-mRNA is stabilized in most types of cancers mediate by binding with HuR, which exports target ARE-mRNA to the cytoplasm. We have developed E4orf6-deficient adenovirus dl355 as an oncolytic virus. E4orf6 is a viral gene essential for viral replication and contributes to viral replication by stabilizing ARE-mRNA in infected cells. Therefore, dl355 can replicate only in cancer cells in which ARE-mRNA is stabilized and lyses the cells. If ARE-mRNA is stabilized not only in PDAC but also in the activated-HPSC, dl355 may be effective for surrounding fibrosis. We found that HuR was relocalized in the cytoplasm of human PDAC and HPSC activated by TGFβ-1. The co-culture enhanced HuR export of MIA PaCa-2 with activated-HPSC. Inhibition of HuR function using CMLD-2 reagent inactivated the growth, invasion and metastasis activities of PDAC. The proliferative and cytopathic effects of dl355 were very high with PDAC and even with activated-HPSC compared to quiescent cells. These results indicate that oncolytic adenovirus dl355 shows potentiality to kill both pancreatic cancer cells and activated-pancreatic stellate cells where an abundance of HuR in the cytoplasm. dl355 has the potential to overcome several critical hurdles proposed by the tumor microenvironment of desmoplastic pancreatic tumors.

PO-018 Inflaming advanced solid tumors including pancreatic cancer using LOAd703, a TMZ-CD40L/4-1BBL-armed oncolytic virus. Jessica Wenthe[1], Emma Eriksson[2], Linda Sandin[3], Tanja Lövgren[2], Justyna Leja Jarblad[3], Hanna Dahlstrand[4], Ulla Olsson-Strömberg[4], Aglaia Schiza[4], Anders Sundin[4], Sandra Irenaeus[4], Eric Rowinsky[5], Gustav Ullenhag[4], Angelica Loskog[6]. [1]Uppsala University, Uppsala, Sweden, [2]Uppsala University, Uppsala, Sweden, [3]Lokon Pharma AB, Uppsala, Sweden, [4]Uppsala University Hospital, Uppsala, Sweden, [5]Lokon Pharma AB, New York, NY, [6]Uppsala University & Lokon Pharma AB, Uppsala, Sweden.

Pancreatic ductal adenocarcinoma (PDAC) is resistant to PD1/PDL1 blocking antibodies. The purpose of this study was to evaluate if immunotherapy with LOAd703, an oncolytic adenovirus armed with trimerized CD40L and 4-1BBL, can sensitize PDAC to mAbs targeting PD1/PDL1. The high content of myeloid-derived suppressor cells (MDCSs) in the tumor microenvironment may in part explain the limited anti-tumor T cell response in PDAC. We have previously shown that gemcitabine can reduce MDSCs in patients with PDAC, with the T cell proliferative capacity remaining intact. Further, LOAd703 has been demonstrated to efficiently and robustly kill PDAC cells and stimulate the maturation of dendritic cells (DCs), which, in turn, induces T cell activation. Patients with advanced PDAC, colorectal, biliary, and ovarian cancer (NCT03225989) were treated with intratumoral injections of LOAd703 combined with appropriate chemotherapy such as gemcitabine ± nab-paclitaxel for PDAC patients, or combined

with gemcitabine if there was no standard of care. Blood sampling was performed for immune cell profiling and anti-adenovirus antibodies (flow cytometry, ELISA). Tumor biopsies were also analyzed for mRNA expression (NanoString; PanCancer Immune Profiling Panel). The study was approved by the ethical review board and the Swedish Medical Products Agency. The dose of LOAd703 was escalated (5x10e10 VP, 1x10e11 VP, 5x10e11VP) in separate cohorts of new patients and administered every other week. To date, blood samples from 23 patients have been analyzed by flow cytometry to profile immune cells. The mean percentages of monocytic- and granulocytic MDSCs, Tregs and M2-like myeloid cells were significantly decreased after treatment initiation. The effector memory (EM) and central memory (CM) CD8+ T cells were significantly increased, while naïve and CM cells were increased among CD4 T cells. Both CD4 and CD8 T cells expressing ICOS were present post-treatment but only CD8 T cells showed significant elevation of PD1. The NanoString data were analyzed using published mRNA immune signatures (bioinformatics). LOAd703 in combination with chemotherapy inflamed tumor lesions as shown by increased markers of the T cell inflamed signature (16 genes), T effector cell signature (19 genes), expanded immune signature (25 genes) and IFNg-related gene signature (12 genes) (week 1 versus week 13). Anti-adenovirus antibody titers increased in all patients after treatment initiation. The antibody levels did not correlate with the dose of virus, radiological response to treatment, nor overall survival. The results presented herein show that LOAd703 combined with chemotherapy enhances immune reactivity in patients with immune cold tumors as demonstrated by increases in EM and CM T cells in the peripheral blood, while suppressive immune cells were decreased. Further, the tumor milieu was inflamed post treatment as shown by mRNA profiling. Hence, LOAd703 may sensitize immune cold tumors to mAbs targeting PD1/PDL1.

PO-019 Reprogramming of naïve B cells in pancreatic cancer subverts humoral immunity. Bhalchandra Mirlekar, Yuliya Pylayeva-Gupta. Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC.

B cells frequently infiltrate human tumors, and the intra-tumoral abundance of plasma cells can correlate with improved patient prognosis. However, many tumors are devoid of plasma B cells, and strategies to enhance anti-tumor B cell responses are needed. We report the existence of a negative regulatory signaling network that reprograms naïve B cells in pancreatic cancer to antagonize anti-tumor plasma B cells. This network is driven by IL-35-mediated STAT3 activation, which directly stimulates upregulation of the pioneer transcription factors Pax5 and Bcl6 in naïve B cells and impedes plasma cell differentiation while simultaneously activating regulatory B cell phenotypes. Significantly, inhibition of Bcl6 reversed this tumor-associated reprogramming of naïve B cells, enabling intra-tumoral accumulation of plasma cells, and reduced tumor growth. Our data provide evidence that B cell dysfunction in cancer involves a potentially targetable suppression program that alters the differentiation potential of naïve B cells.

PO-020 Heating up immune cold pancreatic adenocarcinoma with bioengineered immunotherapy remodels tumor microenvironment and prevents metastasis *in vivo*. Chanthirika Ragulan[1], Patrick Varun Lawrence[1], Hari Ps[1], Krisha Desai[1], Jun Ishihara[2], Anguraj Sadanandam[1]. [1]The Institute of Cancer Research, Sutton, United Kingdom, [2]Imperial College London, London, United Kingdom.

Background: Immunotherapy has resulted in a paradigm shift in the treatment of multiple solid tumors. Still, immunotherapy in unselected pancreatic ductal adenocarcinoma (PDAC) patients has been disappointing. This failure is likely multifactorial and associated with a high immunosuppressive and desmoplastic tumor microenvironment (TME). This unique TME in PDAC leads to various immune escape mechanisms employed by the tumor. While this could be overcome by therapy involving IL12 (or other cytokines) alone, they often fail due to immune-related adverse effects (irAEs). On the other hand, bioengineered collagen-binding domain-linked IL-12 (CBD-IL12; T- cell stimulant that delivers IL12 to collagen-rich regions) is effective in other cancers in pre-clinical settings. Here, we hypothesise that different immune (cold/hot) and collagen content in mice determines their treatment efficacy and irAEs to CBD-IL12 or combination immunotherapy. **Methods:** Three (7947, 7784 and 2334) out of 12 syngeneic orthotopic models of PDAC were classified into immune "hot" ($immune^{hot}$) or "cold" ($immune^{cold}$) with varying collagen levels ($collagen^{high}$ or $collagen^{low}$) using cross-species statistical inference (*in silico* analysis). Mice were treated with vehicle or two bioengineered treatment arms – CBD-IL-12 or CBD-IL-12 + anti-PD1. Tumor growth and metastasis was assessed in an interventional trial. Transcriptome profiling was performed on harvested tumors. **Results:** As predicted by *in silico* analyses, our *in vivo* experiments confirmed 7947 syngeneic model as immune cold ($immune^{cold}$) and resistant to selected stimulatory (GITR) and inhibitory (anti-PD1 or anti-CSF1R+anti-PD1) immunotherapies. To improve the immunotherapy in this $immune^{cold}$ model, we utilised the opportunity that this model has increased collagen ($collagen^{high}$), hence, treating them with CBD-IL12 or combination with anti-PD1 therapy may provide improved treatment efficacy and reduce irAEs. Remarkably, the treatments prevented distant metastasis to the liver by significantly reducing tumor burden ($p<0.05$) and toxicity. The mechanistic analysis demonstrated a cascade of TME changes, including increased CD8 T cells and MHCII+ myeloid cell-based antigen presentation in the treated tumors compared to the control. This increased antigen presentation was associated with macrophage repolarisation from M2 to M1. This was not the case in two other $immune^{cold}/collagen^{low}$ or $immune^{hot}/collagen^{high}$ models. **Conclusion:** Our integrated strategy to pre-select mouse models based on their TME (immune/collagen) profiles improves personalised bioengineered immunotherapy response in immune cold PDAC. Furthermore, the added benefit of preventing metastasis is rather promising and warrants further investigation in aggressive disease like PDAC.

Metabolism

PO-021 Targeting the mitochondrial pyruvate complex to alter metabolic programming in pancreatic cancer. Hassan A. Ali[1], Andrew Metcalfe[2], James T. Topham[2], Cassia S. Warren[2], Joanna M. Karasinska[2], David F. Schaeffer[2], Daniel J. Renouf[2]. [1]University of British Columbia, Vancouver, BC, Canada, [2]Pancreas Centre BC, Vancouver, BC, Canada.

Pancreatic ductal adenocarcinoma (PDAC) can be stratified into distinct transcriptome subtypes, with the 'basal-like' or 'squamous' subtype being associated with worse prognosis, compared to the 'classical' subtype. Our group recently demonstrated that PDAC tumors have unique metabolic transcriptome profiles, and that genes involved in glycolysis and cholesterol synthesis pathways are positively correlated with basal-like and classical gene expression patterns, respectively. The mitochondrial pyruvate complex (MPC) mediates the transport of pyruvate into

the mitochondria which attenuates the effect of glycolysis on tumor progression. The mitochondrial pyruvate carrier 1 (MPC1) gene, which encodes one of two subunits of MPC, is deleted in over 60% of metastatic PDAC and PDAC glycolytic tumors have lowest levels of MPC1 expression. Using PDAC tissue microarrays, we also found that reduced MPC1 protein expression correlates with reduced survival in patients. We hypothesized that targeting MPC1 will alter metabolic reprogramming and may modulate tumor aggressiveness and therapeutic vulnerability in PDAC tumor cells. Genomically and clinically annotated patient-derived tumor organoids (PDOs) were generated from metastatic biopsies from patients enrolled in the PanGen study (NCT02869802). PDOs from both basal and classical tumors were used in the study. In order to investigate glycolysis in PDOs, we adapted the Seahorse Glycolytic Stress Test. Glycolysis, glycolytic capacity and reserve were analyzed in PDOs under basal and treated conditions. To alter MPC1 activity, PDOs were treated for 48 hours with 5uM of UK-5099, an MPC1 inhibitor, or 2.5-5uM SRT1720. SRT1720 is an activator of sirtuin 1 (SIRT1) and the transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator-1α (PGC1-α), which regulates the expression of MPC1. An unpaired t-test with an alpha of 0.05 was used for all statistical analysis. Glycolysis analysis revealed distinct glycolytic profiles in PDOs with differences in glycolytic capacity and reserves trending with different tumor subtypes. Treatment with UK-5099 resulted in an increase in both glycolytic rate and reserve in PDOs from basal and classical tumors. Treatment with SRT1720 resulted in significantly reduced glycolytic rate and capacity. These data suggest that PDAC PDOs exhibit distinct metabolic profiles and that targeting MPC1 can modulate glycolysis in PDOs. Our ongoing efforts aim to further characterize the subtype-specific effect of MPC1 modulators on glycolysis and chemotherapy response in PDAC PDOs.

PO-023 Impaired adipose anabolism drives fat wasting in pancreatic cancer cachexia.
Katherine Pelz, Grace McCarthy, Heike Mendez, Samantha Z. Brown, Jonathan R. Brody, Aaron J. Grossberg. Oregon Health & Science University, Portland, OR.

Purpose/Objectives: The disease associated wasting condition, cachexia, is a common complication of pancreatic ductal adenocarcinoma (PDAC) that impacts quality of life and portends poor survival. Cachexia remains refractory to nutritional supplementation, but the mechanisms underlying this phenotype are unclear. By examining the adipose response to different nutritional contexts in cachectic mice, we sought to understand the relative contributions of enhanced catabolism and impaired anabolism on adipose tissue wasting. **Materials/Methods:** Adult C57BL/6J mice received orthotopic PDAC tumor injections (*Kras^{G12D}; p53$^{R172H/+}$; Pdx1-cre*) or sham injections. Mice were fasted 24 h or fasted and refed 24 h at mid-cachexia time point, and gross fat pads were weighed to assess anabolic potential. 3T3-L1 cells were treated with PDAC cell conditioned media during or after differentiation to model cachexia *in vitro*. Lipolytic rate was quantified as normalized quantity of glycerol released from inguinal (iWAT) and gonadal (gWAT) fat pads *ex vivo* or differentiated 3T3-L1 cells *in vitro*. Lipogenesis was measured *in vitro* using Oil-red-O staining. Adipose tissue mRNA levels were measured using bulk RNAseq and confirmed with qPCR. Malabsorption was assessed via fecal protease activity and total fecal protein and lipid content. **Results:** Adipose tissue in PDAC and control mice were equally sensitive to fasting/food restriction. Both iWAT and gWAT fat pads from PDAC mice showed decreased lipolysis *ex vivo*, associated with decreased lipase (*Atgl & Lipe*) mRNA expression. In the fast/refeed paradigm, PDAC mice were unable to restore both

iWAT and gWAT mass after refeed, in contrast to control mice. We found no evidence of malabsorption at this timepoint by any measure. RNAseq on gWAT revealed 614 differentially expressed genes between PDAC and control mice. Downregulated (n=111) genes were most closely associated with adipogenesis (adj $p<.05$), and expression of adipogenesis master regulators *Pparg* and *Cebpa* reduced in WAT from PDAC mice. Upregulated genes were associated with increased RELA and NFkB activity (adj $p<5x10^{-22}$) and inflammatory response hallmarks. Treatment of 3T3-L1 cells with PDAC conditioned media phenocopied *in vivo* model, showing impaired lipolysis and lipogenesis, associated with decreased expression of lipolytic, adipogenic, and lipogenic genes. **Conclusions:** Adipose tissue wasting in PDAC cachexia can result from impaired anabolism in the absence of enhanced lipolysis or malabsorption. This deficit in adipogenesis is mediated by a cancer cell secreted product and is associated with increased inflammatory signaling in adipocytes, most likely via RELA and NFkB activity. Restoring adipose anabolic potential may provide a novel approach to cachexia treatment in PDAC.

PO-024 Targeting cellular metabolism with CPI-613 sensitizes pancreatic cancer cells to radiotherapy. William A. Hall[1], Husain Y. Khan[2], Mandana Kamgar[1], Susan Tsai[1], Kathleen Christians[1], Douglas B. Evans[1], Philip Philip[2], Callisia Clarke[1], Ben George[1], Beth Erickson[1], Asfar S. Azmi[2]. [1]Medical College of Wisconsin, Milwaukee, WI, [2]Karmanos Cancer Institute, Wayne State University, Detroit, MI.

Novel treatment strategies for pancreatic ductal adenocarcinoma (PDAC) are desperately needed. Local tumor progression is a cause of significant morbidity and mortality in patients with surgically unresectable disease. Often, regional anatomic structures limit the total doses of radiation therapy (RT) that can be safely delivered. Conventional doses of concurrent chemotherapy and RT (chemo-RT) have shown suboptimal results in local control of disease, progression free survival, and overall survival. Therefore, novel and effective approaches to enhance the efficacy of RT are urgently needed to improve overall survival in unresectable PDAC. Metabolic reprogramming enables cancer cells to adjust their metabolism to support increased energy requirements associated with continuous growth and proliferation. Indeed, metabolic reprogramming is a hallmark of PDAC and is associated with increased tumor cell plasticity and chemo-RT resistance. Cancer-cell mitochondria are key regulators of deranged tumor metabolism and have been shown to guide molecular pathways involved in radio-resistance. There is also expanding data that the presence of metabolites modulates the response of cancer cells to RT primarily by impacting the ability to repair DNA. This makes them an optimal candidate for novel radiosensitization strategies, as these characteristics are unique to PDAC cells, and are limited in normal cells. CPI-613, is an analog of lipoic acid which inhibits pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase (α-KGDH), thereby disrupting mitochondrial metabolism leading to selective tumor cell killing. The drug has demonstrated significant clinical activity in patients with metastatic PDAC in combination with standard of care chemotherapies. It remains unknown as to the efficacy in patients treated with concurrent chemo-RT. Here we show that combined treatment of RT (2 and 10 Gy) with CPI-613 (used at 200 and 300 μM) causes superior inhibition of pancreatic cancer cell growth (MTT assay and colony formation assay). In addition, we demonstrate enhanced apoptosis (Annexin V FITC and 7AAD assay) of PDAC cells when treated with a combination of RT and CPI-613. Molecular analysis revealed superior inhibition of PDH and α-KGDH at the protein level.

Targeted metabolomic analysis on PDAC cells post CPI-613-RT treatment revealed alterations in key mitochondrial metabolites, leading to these findings. These results indicate broader target engagement by this combination treatment, indicating the sensitization of CPI-613 treated PDAC cells to radiotherapy at doses as low as 2 Gy. Furthermore, in our preclinical cellular models, a combination treatment of CPI-613 with either Gemcitabine or 5-FU has shown synergistic effects on the proliferation of PDAC cells. Pre-clinical anti-tumor efficacy of the CPI-613-RT and CPI-613-RT-chemo using subcutaneous and orthotopic PDAC models is planned. Our results bring forward a novel combination of CPI-613-RT that warrants further pre-clinical and early phase clinical investigations.

PO-025 Investigating lipid homeostasis in pancreatic ductal adenocarcinoma under tumor-like stress. Xu Han, Michelle Burrows, Celeste Simon, Yanqing Jiang, Brian Keith. University of Pennsylvania, Philadelphia, PA.

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy with a 5-year survival rate of only 10%. Nearly 95% of PDAC harbor oncogenic KRAS, which has been largely “undruggable” in the past. One hallmark of PDAC tumor microenvironment is dense desmoplasia, as a result of abnormal accumulation of extracellular matrix and proliferative fibroblasts. During tumorigenesis, pancreatic stellate cells (PSCs) become activated to a myofibroblast-like phenotype and contribute to the fibrotic environment of PDAC. Desmoplasia-induced hypovascularity severely limits the delivery of oxygen and nutrients. Our laboratory revealed that hypoxia is prominent even at the pancreatic intraepithelial neoplasia (PanIN) stage, which is the dominant precursor lesion of PDAC. Hypoxia inhibits oxygen-dependent processes in cancer metabolism, such as unsaturated lipid biosynthesis which requires oxygen as the terminal electron acceptor. Hypoxia also stimulates exogenous lipid uptake. Previous publications demonstrated that RAS-transformed cells preferentially take up unsaturated lysophosphatidylcholines (LPCs) from culture media. However, under limited nutrient conditions, in the desmoplastic environment, the potential lipid source is not well understood. Interestingly, a lipidomic study of a PSC secretome displayed a significant elevation of LPCs, suggesting that PSCs are the potential lipid source in the PDAC microenvironment. Our data indicate that exogenous unsaturated fatty acids sustain PDAC cell viability under hypoxia and nutrient deficiency. We also find PDAC cell survival under tumor-like stress is enhanced in PSC conditioned medium, but not in delipidated conditioned medium. Strikingly, we show that cancer-associated fibroblasts are not experiencing hypoxic stress as much as malignant epithelium cells *in vivo* by analyzing single-cell RNA-seq data and IHC, which suggests the capability of cancer-associated fibroblasts in providing unsaturated lipids to PDAC cells. Based on our lipidomic mass spectra of PSC conditioned medium, we identify that LPCs are actively imported by PDAC cells and prove that unsaturated LPCs maintain PDAC cell survival under nutritional stress. Our overall hypothesis is that activated PSCs support PDAC cell survival by providing unsaturated lipids, particularly LPCs, and inhibition of unsaturated lipid uptake through impeding lipid transporter activity or LPC synthesis by PSCs could potentially induce PDAC cell death under metabolically challenging conditions. We will hinder possible pathways responsible for LPC uptake in PDAC cells and generate *in vivo* models to achieve therapeutic benefits of PDAC. In summary, our research aims to demonstrate the metabolic crosstalk between PSCs and PDAC cells under stress conditions. The key advance of this study is that activated PSCs suppress PDAC cell death by supplying tumor cells with unsaturated LPCs for

maintaining lipid homeostasis, in a hypoxic and nutrient-poor environment. Our findings will reveal a novel metabolic target for developing combinatorial therapy of PDAC.

PO-026 CircMYOF acts as a miR-4739 sponge to promote progression and facilitate glycolysis via VEGFA/PI3K/AKT pathway in pancreatic ductal adenocarcinoma. Dandan Zheng[1], Xianxian Huang[2], Juanfei Peng[1], Yanyan Zhuang[1], Yuanhua Li[3], Junchi Qu[1], Shineng Zhang[1], Fengting Huang[1]. [1]Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China, [2]the Eighth Affiliated Hospital, Sun Yat-sen University, Shenzhen, China, [3]Tungwah Hospital of Sun Yat-sen University, Dongguan, China.

Emerging evidences has revealed that circular RNAs (circRNAs) participate in the initiation and development of pancreatic ductal adenocarcinoma (PDAC), a deadly malignancy with extremely low 5-year survival rate. Reprogrammed glucose metabolism is a key feature of tumor development, including PDAC. In this study, we aimed to investigate the role of circRNAs in reprogrammed glucose metabolism in PDAC. RNA sequencing under various glucose incubation circumstance was performed. A new circMYOF was identified. Sanger sequencing and RNase R treatment confirmed its circular RNA characteristics. Real time PCR indicated that it was overexpressed in PDAC tissues and cell lines. Gain-of and loss-of function assay implied that circMYOF promoted progression in PDAC. Mechanically, RNA pull down and luciferase reporter assay suggested that circMYOF, exhibiting as a competing endogenous RNA (ceRNA) for miR-4739, facilitated glycolysis via VEGFA/PI3K/AKT pathway. Taken together, our finding indicates that circMYOF may be a potential biomarker and therapeutic target for PDAC patients.

PO-027 Investigating unfolded protein responses in pancreatic ductal adenocarcinoma under lipid imbalance. Yanqing (Christine) Jiang, Xu Han, Michelle Burrows, Carson Poltorack, Celeste Simon, Brian Keith. University of Pennsylvania, Philadelphia, PA.

Pancreatic ductal adenocarcinoma (PDAC) is the 4th leading cause of cancer-related death with a five-year survival rate of only 10%. A major feature of PDAC tumor microenvironment (TME) is desmoplasia, which are dense fibrotic and inflammatory stromal regions constituting the PDAC TME. This desmoplastic reaction results in poor intratumoral perfusion and elevated interstitial fluid pressure, creating regions with severe oxygen and nutrient deprivation. One result is a significant deficiency in unsaturated fatty acids (UFAs) because the *de novo* synthesis of UFAs requires molecular oxygen. Our data indicate that PDAC cells experience endoplasmic reticulum (ER) stress and trigger a subsequent unfolded protein response (UPR) under tumor-like stress, which can be mitigated by UFAs. The UPR regulates an intricate signaling network mediated by three major stress sensors: Inositol-requiring enzyme 1a (IRE1a), PKR-like ER kinase (PERK) and activating transcription factor 6a (ATF6a). UPR output can be either cytoprotective or cytotoxic depending on tumor types and the severity of stress. Our data suggest that B-I09, a selective IRE1 RNase inhibitor, impairs PDAC cell viability under stress conditions, indicating a pro-survival role for IRE1a. Previous studies showed that MYC-driven cancer cells are highly reliant on UPR pathways. Therefore, we hypothesize that the two major PDAC transcriptional subtypes “Classical” and “Basal-like” may have distinct dependencies on UPR pathways, with the Basal-like subtype being more dependent due to an enrichment of MYC gene signatures. Overall, we hypothesize that IRE1a, PERK and/or ATF6a play(s) an oncogenic

role in PDAC tumorigenesis due to lipid saturation abnormalities, and the Basal-like PDAC subtype is more sensitive to inhibition of UPR pathway(s). We are evaluating the role of IRE1a, PERK and ATF6a using pharmacologic inhibition and genetic knockdown *in vitro* and *in vivo* under UFA deprived conditions. We will also evaluate PDAC intracellular and interstitial fluid lipidomics using LC-MS. In addition, we will assess whether hypoxic PDAC cells experience ER stress *in vivo* due to UFA deprivation using Stimulated Raman scattering (SRS) microscopy. To explore the subtype-specific dependency on the UPR pathways, we are querying a UPR gene signature in different tumor cell clusters using single cell RNA-seq datasets of patient samples. In addition, we will employ an isogenic system for PDAC subtypes and examine subtype-specific responses to UPR inhibition *in vitro* and *in vivo*. Our goal is to better understand UPR pathways in PDAC cells as a survival mechanism in response to the "lipotoxicity" induced by ER membrane saturation, with a consideration of intratumoral heterogeneity of PDAC cell subtype.

PO-028 Pancreatic ductal adenocarcinoma is dependent on an unconventional pathway for polyamine synthesis. Min-Sik Lee[1,2,3], Insia Naqvi[1], Courtney Dennis[4], Lucas Dailey[4], Alireza Lorzadeh[5], Tamara Zaytouni[1], Ashley Adler[1,3], Daniel S. Hitchcock[4], Lin Lin[1], Unmesh Jadhav[5,6], Clary B. Clish[4], and Nada Y. Kalaany[1,2,3]. [1]Division of Endocrinology, Boston Children's Hospital, Boston, MA, [2]Department of Pediatrics, Harvard Medical School, Boston, [3]Broad Institute of MIT and Harvard, Cambridge, MA, [4]Metabolomics Platform, Broad Institute of MIT and Harvard, Cambridge, MA, [5]Department of Stem Cell Biology and Regenerative Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, [6]Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA.

Targeting altered metabolism in pancreatic ductal adenocarcinoma (PDAC) has been an area of extensive investigation for over a decade now. A major hurdle however, for most anti-tumor metabolic strategies, is the high risk for toxicity, given the essential roles of metabolic pathways in the maintenance of normal tissue homeostasis. Indeed, this has been the case for targeting polyamines in cancers. Polyamines are small, highly positively charged molecules involved in multiple fundamental processes of cell growth and survival, including the synthesis of nucleic acids, modifications of chromatin structure, gene transcription and mRNA translation. Polyamine levels are significantly increased in many cancers, including PDAC. Prior anti-tumor strategies focused on pharmacological inhibition of the rate-limiting enzyme of polyamine synthesis, ornithine decarboxylase (ODC1) with little success, partially due to risk of harming normal tissues at higher drug doses. In this project, using both *in vitro* and *in vivo* mouse models of PDAC, we identified a dependency of PDAC on an unconventional way for the synthesis of the polyamine precursor ornithine, specifically from glutamine via ornithine aminotransferase (OAT); this is in contrast to its synthesis in most adult normal tissues from arginine via arginase (ARG) activity. We also identified potential key players mediating the induction of this metabolic pathway via KRAS, the main oncogenic driver in PDAC and are currently characterizing the downstream effects of polyamines on pancreatic tumor cells. The high dependency of PDAC, compared to normal tissue, on *de novo* ornithine synthesis via OAT provides an attractive therapeutic window for treating pancreatic cancer patients with minimal toxicity.

PO-029 Pancreatic cancer-associated cachexia as a 3-stage systemic disease with changes in body composition, tissue-specific wasting across time and alterations in glucose metabolism. Blanca Majem[1], Insia Naqvi[1], Courtney Dennis[2], Lucas Dailey[2], Clary B. Clish[2], Nada Kalaany[1]. [1]Boston Children's Hospital, Harvard Medical School, Boston, MA, [2]Metabolomics Platform, Broad Institute of MIT and Harvard, Cambridge, MA.

Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease that rapidly deteriorates the organism resulting in <10% 5-years survival in humans. Cancer-associated cachexia (CAC) is a multi-organ complex syndrome that accompanies PDAC in 85% of the cases. CAC is often characterized by the loss of body weight, including loss of muscle and fat tissues. Chronic inflammation, myokines and adipokines (released from muscle and fat, respectively), together with few recent tumor-derived factors, have been shown to collectively induce some of the metabolic changes in peripheral tissues that lead to wasting. Those include the synthesis of acute phase proteins in the liver, lipolysis and browning of the white adipose tissues (WAT), and protein degradation in the skeletal muscle. However, how these changes affect systemic metabolism, such as amino-acid utilization for liver gluconeogenesis, the systemic consequences of increased free fatty acids from lipolysis and their final destination for b-oxidation, or the rewiring of glucose metabolism, are processes not fully understood during cachexia. Here we established a 3-stage model of cachexia progression, including pre-, early- and late-CAC stages, in a doxycycline inducible murine model of PDAC (p48(Cre/+);tetKRAS(LSL/+);p53(fl/fl)), in both genders. We have monitored body weight loss, changes in body composition using DEXA scan, food intake and survival, across time since the start of doxycycline administration (i.e. tumor initiation). We have submitted these mice to metabolic cages aiming to distinguish in which of the 3 stages fat consumption starts, as well as monitored the weight of 8 tissue types (pancreas, liver, iWAT, eWAT, brown adipose tissue, quadriceps, gastrocnemius and soleus) over time. Overall, we have defined pre-CAC as “weight-gaining stage, before body weight peaks”, early-CAC as <10% and late-CAC as >10% of body weight loss, together with specific changes in body composition in each stage, food intake only happening at the very end stage, while all non-tumoral tissues present significant reduced weight at early stages. We have performed metabolomics and lipidomics in all peripheral tissues, aiming to identify changes that occur not only at early- but also at the pre-CAC stages, when pancreatic tumors already weighed more than double of the normal pancreas. In addition, glucose tolerance test analyses showed that tumor-bearing mice cleared the glucose more rapidly than control mice, even at pre-CAC stages, opening new avenues to continue studying glucose metabolism in the periphery such as increased liver gluconeogenesis and peripheral insulin resistance. Together, these results allowed us to establish a murine model to study cachexia in 3 stages, similar to what happens in humans. Furthermore, metabolomic data from the peripheral tissues, tumor interstitial fluid (TIF) and plasma, will allow us to identify the metabolic landscape of the entire organism throughout the progression of the disease, and potentially propose new therapeutic windows to target or prevent wasting in cancer.

PO-031 Lysosome inhibition overcomes resistance to CDK4/6 inhibition in PDA. Dilru Silva, Conan Kinsey, Martin McMahon. Huntsman Cancer Institute, University of Utah, Salt Lake City, UT.

The current FDA-approved chemotherapies for patients with Pancreatic Ductal Adenocarcinoma

(PDA) demonstrate substantial toxicities with only a modest survival benefit highlighting the urgent need to better understand the molecular mechanisms driving PDA and how they interact in order to develop more precise and less toxic pathway-targeted therapies. PDA often harbors oncogenic mutations in *KRAS* and *CDKN2A* that drive a dysregulated cell cycle through activation of CDK4/6 (Cyclin-Dependent Kinases 4 and 6), which mono-phosphorylate RB1 to govern progression through G0◊G1◊S phases. Currently, there are three FDA-approved CDK4/6 inhibitors, palbo-, abema-, and ribociclib (X-ciclibs), that are predicted to have anti-cancer activity in PDA cells. However, the benefits of X-ciclib treatment in PDA patients are largely underwhelming. We found that inherent resistance to X-ciclib treatment *in vitro* is due to compensatory activation of protective autophagy, a critical lysosomal degradation process that generates a source of nutrients during periods of cellular stress. We observed that X-ciclib treatment induces autophagic flux in PDA cell lines. This pro-cancer process can be targeted with the lysosomal inhibitors, chloroquine and hydroxychloroquine. The addition of chloroquine sensitizes PDA cells to X-ciclib treatment, resulting in diminished proliferative ability. Additionally, a late-stage PDA patient was treated with the combination of an X-ciclib and hydroxychloroquine and displayed a dramatic decrease in the bloodborne PDA biomarker, Cancer Antigen 19-9. These data may suggest a novel combination treatment strategy for late-stage PDA patients, but the molecular mechanisms underlying are yet to be studied in PDA. Future work will explore the role RB1 plays in CDK4/6 regulated autophagy by investigating how phosphorylation at distinct RB1 sites modulates autophagy-related gene expression and the extent to which mTORC1 deactivation is necessary for CDK4/6 regulated autophagy.

Microbiome

PO-033 Bacterial cytotoxin therapy limits tumor growth for pancreatic ductal adenocarcinoma. Amanda R. Decker[1], Tetsuhiro Harimoto[2], Steve A. Sastra[1], Tal Danino[2], Kenneth P. Olive[1]. [1]Columbia University Medical Center, New York, NY, [2]Columbia University, New York, NY.

Treating pancreatic ductal adenocarcinoma (PDAC) with systemic chemotherapeutic drugs has remained a challenge, due in part to the hypovascularized and poorly perfused nature of PDAC tumors. Limited blood flow within the tumor tissue creates an extremely hypoxic microenvironment and impedes the accumulation of drugs. Moreover, local immunosuppression in PDAC has so far limited the efficacy of immunotherapy approaches. However, these very features that have interfered with systemic therapy in PDAC (hypoperfusion, hypoxia, and immunosuppression) are potential advantages for the use of bacterial therapies. Bacteria have been used as a directed cancer therapy for over 100 years, starting with Dr. William Coley's use of heat killed bacteria (Coley's Toxin) against sarcomas in 1893. Recent developments in the field of synthetic biology have made it possible to engineer complex logical circuits into bacteria, enabling the manufacture of anticancer therapies directly within the tumor parenchyma. Bacteria can actively migrate through tissues, they can thrive in hypoxic microenvironments, and they benefit from the local immune suppression. We have therefore worked to develop novel bacterial strains for targeting PDAC. We began by testing a range of bacteria-derived toxins that could be used as a payload to target PDAC. These toxins were produced by an engineered strain of a non-toxic, probiotic *E. coli Nissle 1917*. We identified four pore-forming toxins that significantly reduced viability of the cells compared to bacterially produced GFP: hemolysin E,

heat stable enterotoxin, magainin, and theta toxin. We then performed a secondary screen using a novel PDAC explant model system developed in our lab, in which thick slices of murine or human PDAC are culture intact for up to 7 days. Consistent with the monolayer screen, two of the candidate compounds, heat stable enterotoxin and theta toxin, significantly increased tissue death compared to non-toxic GFP-producing bacteria. Finally, we delivered live bacteria producing either toxins or GFP into KPC mouse tumors through intratumoral injection. While GFP-producing strains did not induce a change in tumor growth kinetics, theta toxin treatment demonstrated an immediate, prolonged stabilization of tumor volume for more than a month. Histological analyses of treated tumors demonstrated that diffuse populations of bacteria co-localized with regions of tumor necrosis and cell death. Most interestingly, while there was minimal spread of bacteria to healthy non-target tissues, translocation of the bacteria did occur to regions of liver metastases and secondary papilloma tumors, suggesting a mechanism for diffuse treatment of known and unknown metastases following the initial treatment of the primary tumor. Together these studies demonstrate that cytotoxic bacterial therapy is an effective candidate strategy to circumvent the difficulties in systemic treatment of PDAC.

Other

PO-034 CPSF3 inhibition halts pancreatic cancer cell proliferation by limiting core histone supplies. Abdulrahman A. Alahmari[1], Carla Schwarz[2], Emily Paterson[2], Swati Venkat[2], Arwen Tisdale[2], Michael E. Feigin[2]. [1]Roswell Park Comprehensive Cancer Center, Amherst, NY, [2]Roswell Park Comprehensive Cancer Center, Buffalo, NY.

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies with few effective treatment options. Therefore, the need for new therapeutic interventions is urgent. Recently, we have revealed that the gene regulatory process alternative polyadenylation (APA) is highly dysregulated in PDAC patients. APA is a co-transcriptional process that generates distinct transcript isoforms with different 3' untranslated region (UTR) length, which regulates mRNA stability and gene and protein expression. We found that APA promotes dysregulation of PDAC-promoting genes, predicts poor prognosis in PDAC patients and identified a novel APA-regulated drug target (Venkat et al., Genome Research, 2020). These findings raise the question of whether targeting APA could be a viable therapeutic strategy for PDAC treatment. APA is controlled by a large protein complex assembled on the pre-mRNA, including the cleavage and polyadenylation specificity factor 3 (CPSF3). CPSF3 is an endonuclease that cleaves the pre-mRNA before addition of the polyA tail. Notably, CPSF3 is also a subunit of the histone pre-mRNA processing complex and is critical for the cleavage and processing of core histones. Recently, it was determined that JTE-607, a drug with a long history of safety in humans but no known mechanism of action, is a CPSF3 inhibitor. Subsequently, CPSF3 has been shown to be required for growth of acute myeloid leukemia and Ewing sarcoma cells. However, whether CPSF3 is a potential target in adenocarcinoma is yet to be explored. In PDAC patients, CPSF3 is highly expressed and is associated with unfavorable prognosis. Knockdown or inhibition of CPSF3 decreases PDAC cell proliferation and clonogenicity while having no effect on the growth of non-transformed pancreatic cell lines. Cell cycle analysis and BrdU incorporation assays determined that CPSF3 inhibition arrests cells in early S-phase of the cell cycle. As maintenance of high histone levels are important for the packaging of newly synthesized DNA and progression through the cell cycle, we hypothesized that CPSF3 inhibition decreases PDAC

cell proliferation by reducing histone levels. Indeed, CPSF3 inhibition decreased core histone protein levels in a time- and dose-dependent manner. High throughput screening revealed a synergistic effect between JTE-607 and Abexinostat, an HDAC inhibitor, suggesting a novel therapeutic strategy. Therefore, we identify CPSF3 as a druggable target in PDAC and reveal a novel mechanism by which CPSF3 disruption attenuates cell proliferation.

PO-036 LP184, a novel alkylating agent, is highly effective in pancreatic cancers with DNA damage repair defects. Diana Restifo[1], Aditya Kulkarni[2], Caleb Schimke[2], Joseph McDermott[2], Umesh Kathad[2], Kishor Bhatia[2], Panna Sharma[2], Igor Astsaturov[1]. [1]Fox Chase Cancer Center, Philadelphia, PA, [2]Lantern Pharma, Dallas, TX.

Biomarker-based chemotherapy with increased efficacy and prolonged disease-free survival are urgently needed in pancreatic cancer (PDAC). A 15-20% subset of PDAC tumors carry mutations in DNA repair pathway (BRCA1/BRCA2/PALB2/RAD51/ATM/FANCD2). Additionally, mutations in nucleotide excision repair (NER) genes (ERCC2/3/4/5/6) have been reported in ~5% of PDAC. LP184 is a novel synthetic small molecule acylfulvene analog. Using precise synthetic chemistry, we determined that only a negative enantiomer of LP184 is converted to an active alkylating agent in the strict dependency on oxidoreductase, prostaglandin reductase 1 (PTGR1). By computational analyses, we demonstrate a strong positive correlation of LP184 sensitivity with PTGR1 transcript levels ($r=0.89$, $p<10^{-15}$) in a broad panel of cancer cell lines. Once activated by PTGR1, highly reactive LP-184 nucleophile creates covalent DNA adducts that are selectively repaired via Nucleotide Excision Repair (NER) mechanism coupled to transcription (TC-NER) and/or homologous recombination (HR). We reasoned that mutation or expression driven TC-NER and HR deficiency would predispose PDAC cells to increased sensitivity to LP184. To test the idea of LP184 activity in DNA repair-deficient tumors, we evaluated LP184 chemosensitivity in genetically defined PDAC models in vitro, ex vivo, and in xenografts. Testing in six different pancreatic cancer cell lines (Capan-1, CFPAC-1, Panc1, MiaPaCa2, Panc03.27 and BxPC-3) resulted in very potent inhibition with LP184 IC50 values ranging from 114 to 182 nM. In this cell line panel, LP184 sensitivity correlated negatively with transcript levels of an NER pathway gene ERCC8 ($r = -0.94$). In comparison to these PDAC cell lines, a normal pancreatic epithelial cell line HPNE was 3-6 times less sensitive to LP184 (IC50 670 nM). Ex vivo cultures of 4 out of 5 low-passage patient-derived xenografts with HR deficiency showed nanomolar sensitivity to LP184 with IC50s ranging from 45 to 270 nM. These tumor graft models which were at least 6 times less sensitive to olaparib in the same assay. Depletion of ERCC4 enhanced sensitivity to LP184 about 2-fold relative to the parental cell line. To define PTGR1 as a biomarker for LP184 activity, we used CRISPR/Cas9-mediated gene editing to deplete PTGR1 expression. We found PTGR1-null Capan-1 cell line-derived xenografts were poorly sensitive to LP184, whereas PTGR1-expressing xenografts showed near complete tumor regression in all LP184 treated animals with 109% tumor growth inhibition relative to the control group in this study. Furthermore, PTGR1 depleted cells were completely resistant to LP184 in vitro. Our preclinical data demonstrate that PDAC models carrying a range of DNA repair pathway mutations are highly sensitive to LP-184 in vitro and in vivo. Increased PTGR1 expression is a validated biomarker for LP184 cytotoxicity, and is the exclusive convertase of LP184 to an active alkylator drug. We anticipate LP184 will extend the therapeutic opportunities to a large subset of PDAC patients carrying these genetic alterations.

PO-037 Development of an RGD CRISPR-modified *Clostridium novyi* NT spores as an intravenous oncotherapy. Kaitlin M. Dailey[1], Krysten Vance[2], Kyle McAndrews[3], Reed I. Jacobson[4], Jandro Delgado[4], Paige R. Johnson[5], Taylor M. Woolery[5], Megan Orr[6], Jiha Kim[7], Sanku Mallik[5], Kenneth W. Bayles[8], Michael A. Hollingsworth[2], Amanda E. Brooks[9]. [1]Eppley Institute for Cancer Research, University of Nebraska Medical Center, and Cell and Molecular Biology Program, Pharmaceutical Sciences Department, North Dakota State University, Omaha, NE, [2]Eppley Institute for Cancer Research, University of Nebraska Medical Center, Omaha, NE, [3]Eppley Institute for Cancer Research, University of Nebraska Medical Center, Omaha, Omaha, NE, [4]Department of Biological Sciences, North Dakota State University, Fargo, ND, [5]Cell and Molecular Biology Program, Pharmaceutical Sciences Department, North Dakota State University, Fargo, ND, [6]Department of Statistics, North Dakota State University, Fargo, ND, [7]Cell and Molecular Biology Program, Pharmaceutical Sciences Department and Department of Biological Sciences, North Dakota State University, Fargo, ND, [8]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, [9]Cell and Molecular Biology Program, Pharmaceutical Sciences Department, North Dakota State University, Fargo, ND, and Department of Research and Scholarly Activity, Rocky Vista University, Ivins, UT.

The efficacy of current oncotherapeutics is largely limited by an inability to access avascular tissues, which is in part responsible for forty years of stagnant pancreatic cancer statistics where the median survival remains a mere six months. Oncolytic bacteria such as *Clostridium novyi*-NT overcome this challenge with its ultrasensitive, innate affinity for hypoxic/necrotic areas found at the center of solid tumors and their metastases. While preclinical and clinical data from intratumoral injections of *C. novyi*-NT are promising, many tumors are inaccessible to such injections. Preclinical trials of analogous IV injections have uncovered other obstacles such as rapid clearance of *C. novyi*-NT by the immune system independent of septic complications. To mitigate rapid clearance, CRISPR/Cas9n was used to genetically modify a non-toxic form of *C. novyi*-NT to express a tumor targeting RGD peptide on the spore surface. Through this novel, first of its kind, methodology, spores with stronger affinity to a surface coated with the targeted binding partner of RGD, a_Vb_3 integrin, have been generated. Importantly, there was no statistically significant difference in the genetically modified spore's capacity for sporulation or germination when compared to unmodified *C. novyi*-NT spores, nor was a difference in lytic capacity observed, suggesting no relevant off-target effects from genomic modification. Biodistribution and efficacy of non-toxic RGD-modified spores was evaluated in an immunocompetent, syngeneic, pancreatic cancer murine model. Ongoing efforts to characterize the biodistribution and efficacy of the intravenously injected RGD-modified *C. novyi*-NT include the application of multiplex immunofluorescence, laser microdissection, and live, whole animal imaging. Supported as a pilot project by funds from NIH COBRE grant 1P20GM109024, Doctoral Dissertation Funds to KMD from NDSU, and by discretionary funds from investigators at UNMC.

PO-038 LAMC2: New player in stemness and tumor progression in pancreatic cancer. Donatella Delle Cave[1], Tea Teresa Iavazzo[1], Maria Mangini[2], Gennaro Andolfi[1], Teresa Pirozzi[1], Annalisa Di Domenico[1], Annachiara De Luca[2], Enza Lonardo[1]. [1]Institute of Genetics and Biophysics 'Adriano Buzzati-Traverso' (IGB), CNR, Naples, Italy, [2]Institute of Biochemistry and Cellular Biology, National Research Council of Italy, Naples, Italy.

Pancreatic ductal adenocarcinoma (PDAC) is a devastating and essentially incurable disease, typically characterized by high chemoresistance and metastatic spread attributable to cancer stem cells (CSCs). This subpopulation is critical for tumor initiation and recurrence, but the mechanism through which they acquire metastatic traits is not well understood. Hence, targeting the CSC niche and their plasticity could be a complementary therapeutic strategy against cancer. Laminin subunit-γ-2 (LAMC2) is an epithelial basement membrane protein, which controls cell motility and adhesion and is widely expressed in the majority of human tumors. However, its role in PDAC remains largely unknown. In several patient cohorts we observed that high levels of LAMC2 significantly correlated with shorter overall survival. In addition, the tissue microarray analysis on PDAC sections revealed prognostic significance of LAMC2 expression in tumor with high grade of aggressiveness (i.e., G2 and G3). To determine the role of LAMC2 in sustaining tumorigenicity, we knocked down it in patient-derived xenografts (PDX) cells using lentiviral shRNA constructs. The silencing of *LAMC2* resulted in decreased self-renewal, invasiveness, tumorigenicity and gemcitabine resistance both *in vitro* and *in vivo*. To identify or track the LAMC2 tumor cell population in an intact environment we engineered primary PDAC cells that carry EGFP cassette knocked in the *LAMC2* locus through the CRISPR-Cas9 technique. Analysis of LAMC2-EGFP^{+} cells isolated from tumor demonstrated that these cells express a gene program similar to that of highly metastatic stem cells and that they initiate and propagate both the primary tumor and the metastasis to recipient mice very efficiently compared to their counterpart. In conclusion, we identified a highly metastatic subpopulation of cancer stem cells, characterized by high levels of LAMC2. Strategies aimed at targeting the LAMC2 population may be effective in reducing tumor aggressiveness in combination with conventional therapy.

PO-039 Antiproliferative activity of inhibitors of RAD51, singly and in combination with chemotherapy drugs, against pancreatic cancer cell lines. Peter Ferguson[1], Mark D. Vincent[1], Yousef Najajreh[2], Brian Shilton[3], Stephen Ritter[3], Rima Al-awar[4], Richard Marcellus[4], Mohammed Mohammed[4], Methvin Isaac[5], James Koropatnnick[4]. [1]London Health Sciences Centre, London, ON, Canada, [2]Al Quds University, Jerusalem, Palestinian Territory, [3]Western University, London, ON, Canada, [4]Ontario Institute for Cancer Research, Toronto, ON, Canada.

Despite the innovations in chemotherapeutic treatment of pancreatic cancer, the generally poor outcome of this disease begs the search for novel targets. RAD51 is a critical component of homologous recombination DNA repair, binding with BRCA2 and forming polymeric filaments essential for its function. RAD51 is often elevated in cancer cells compared to normal cells, particularly in pancreatic cancer: RAD51 is a negative prognostic biomarker and promotes tumor cell proliferation in that disease (Cancer Cell Int 19: 356, 2019; doi: 10.1186/s12935-019-1077-6). Therefore, RAD51 is an attractive target for anticancer treatment in that its elevated level and activity in tumor cells provide a potential level of selectivity for such an agent, increasing the therapeutic index. We demonstrated previously that novel inhibitors of RAD51, IBR2 [2-(Benzylsulfonyl)-1-(1H-indol-3-yl)-1,2-dihydroisoquinoline] and IBR120 (an isoindolinyl derivative of IBR2) (Eur J Med Chem. 96:196-208, 2015; doi: 10.1016/j.ejmech.2015.04.021), not only inhibited proliferation of a range of tumor cell lines at micromolar concentrations but also acted in combination with commonly used cytotoxic chemotherapy and molecularly targeted drugs to synergistically inhibit proliferation (J Pharmacol Expt Ther, 364: 46-54, 2018; doi.org/10.1124/jpet.117.241661). To improve ADME properties, enhance activity, and

increase potential to selectively target RAD51, advanced computational tools and rational approaches were employed to predict possible modification to the IBR chemical backbone. This effort resulted in discovery of the compound JKYN-1. As a model system in which to test the activity of JKYN-1 *in vitro,* human pancreatic cancer cell lines PANC-1, Capan-1 and Capan-2 were used. All lines have mutant K-Ras and p53, and Capan-1 has mutant BRCA2 (https://www.cancer.gov/research/key-initiatives/ras; https://web.expasy.org/cellosaurus). JKYN-1 or its water-soluble methylsulfonate salt (JKYN-1-mesylate) inhibit proliferation of PANC-1 cells approximately 5 times more effectively than IBR120 and inhibit Capan-1 and Capan-2 cells even better. Against PANC-1, JKYN-1-mesylate inhibits proliferation synergistically in combination with afatinib (inhibitor of epidermal growth factor receptor) or with IBR120, and is additive with gemcitabine. Biochemical characterization of the interaction between RAD51 and these inhibitors demonstrated the following: (1) IBR2 and IBR120 promote disassembly of RAD51 multimers in an *ex vivo* native polyacrylamide gel electrophoresis assay using clonal constructs of RAD51, and (2) JKYN-1 binds to RAD51 with higher affinity than IBR120 and B02, a commercially available RAD51 inhibitor, in a surface plasmon resonance spectroscopy assay. Therefore, JKYN-1 is a potential novel small molecule therapeutic agent for treatment of pancreatic cancer, both as a single agent and in combination with standard chemotherapy drugs.

PO-040 Nischarin is expressed in pancreatic ductal adenocarcinoma and is a potential target for drug repurposing. Jelena Grahovac[1], Marijana Pavlovic[1], Marija Ostojic[1], Kristina Zivic[1], Daniel Galun[2], Tatjana Srdic-Rajic[1]. [1]Institute for Oncology and Radiology of Serbia, Belgrade, Serbia, [2]School of Medicine, University of Belgrade; First Surgical Clinic, Clinical Center of Serbia, Belgrade, Serbia.

The objective of this study was to examine the expression of Nischarin (NISCH) in pancreatic ductal adenocarcinoma (PDAC), and its potential as a target for drug repurposing. NISCH has been described as a tumor suppressor in breast and ovarian cancers, and there are several clinically approved agonists for this receptor. NISCH has also been reported as a scaffolding protein with a role in cell adhesion, invasion and metabolism – aspects of the PDAC biology that are of interest for therapeutical intervention. We examined mRNA and protein expression of NISCH in publicly available datasets, patient tumor samples, representative PDAC cell lines and patient-derived stellate cells. We found that NISCH is a prognostic marker in PDAC patients and that it is expressed in both cancer cells and cancer-associated stroma. Gene set enrichment analysis showed that NISCH expression was associated with the regulation of cell motility, cell cycle and energy metabolism. Indeed, NISCH knockdown in cancer cells induced reorganization of the actin cytoskeleton. Next, we tested the effects of three clinically approved NISCH agonists (rilmenidine, clonidine and moxonidine) on cancer cells and cancer-associated fibroblasts (CAFs). We found that rilmenidine, agonist with the highest affinity for the NISCH receptor, had the most potent effect. Rilmenidine impaired cancer cell adhesion, limited migration and decreased production of pro-metastatic cytokines and extracellular matrix deposition in cancer cell-CAF co-cultures. Our study lays a ground for more extensive examination of the role of NISCH in PDAC and implies that NISCH agonists may be good candidates for drug repurposing in this type of cancer.

PO-041 Systemic screening of gene delivery methods in pancreatic ductal adenocarcinoma cells. Dmytro Grygoryev[1], Taelor Ekstrom[1], Jason M. Link[2], Rosalie C. Sears[2], Jungsun Kim[1]. [1]Cancer Early Detection Advanced Research Center, Knight Cancer Institute, Portland, OR, [2]Oregon Health & Science University, Portland, OR.

Deaths in the United States due to Pancreatic Ductal Adenocarcinoma (PDAC) has risen steadily since 1990, and PDAC is expected to be the second leading cause of cancer death by 2025. Such a dismal prognosis is mainly attributed to the fact that tumors are detected too late for an effective treatment. Additionally, even when detected early, PDAC is challenging to treat with existing treatment options. Thus, there is a need for improved treatment strategies for pancreatic cancer patients. Great efforts have been made to develop and test new targets in various model systems such as tumor cell lines, tumor organoid models, and patient-derived xenografts (PDXs). We previously demonstrated a proof-of-principle of cellular reprogramming to pluripotent state to model PDAC progression and utilized it as a discovery tool for early detection markers for PDAC. However, reprogramming efficiency was remarkably low and it is, at least in part, attributed to uneven gene delivery efficiencies across cell types in human pancreatic normal and cancer cells. Intriguingly, classical ductal cell types were more resistant to transduction with conventional VSV-G-pseudotyped lentivirus (LeV) than squamous subtype pancreatic cancer. To better understand the susceptibility of different types of pancreatic cancer cells to viral transduction and improve the gene delivery efficiency, we analyzed transduction of LeV, Sendai Virus (SeV), and episomal vector transfection efficiencies in classical ductal and squamous cell types. We used human pancreatic duct epithelial and foreskin fibroblast cell lines as controls. We found that transduction efficiency of LeV in both types of PDAC cell lines are significantly lower compared to control cell lines and considerably higher in squamous type compared to classical ductal type. In contrast, transduction efficiency of SeV was similar for both classical ductal and squamous types of PDAC cell lines and significantly higher compared to LeV efficiency (90% vs 5-25 % at MOI 1). We also found that nucleofection transfection efficiency of episomal vector is significantly higher in squamous cell type compared to classical ductal cells (67 % vs 55 %). This is significantly higher than transduction efficiency of LeV (25 and 5 % at MOI 1 correspondingly) but lower compared to SeV transduction efficiency. Thus, both SeV and nucleofection delivery methods show higher efficiency compared to LeV and can be successfully used as gene delivery methods in PDAC cell lines. By completing this study, we can provide a tailored gene delivery method for pancreatic cancer, and this information can be harnessed for cellular reprograming of human PDAC as well as testing newly developing targets in other model systems *in vitro* and *ex vivo*.This work is supported by OHSU/CEDAR project award 68182-939-000.

PO-043 Cytidine deaminase protects pancreatic cancer cells from replicative stress and drive response to DNA-targeting drugs. Audrey Lumeau[1], Nicolas Bery[1], Cyril Ribeyre[2], Samad Elkaoutari[3], Guillaume Labrousse[1], Miguel Madrid-Mencia[1], Vera Pancaldi[1], Marie-Jeanne Pillaire[4], Valérie Bergoglio[5], Nelson Dusseti[3], Jean-Sébastien Hoffmann[6], Louis Buscail[7], Malik Lutzmann[2], Pierre Cordelier[1]. [1]Cancer Research Center of Toulouse, Toulouse, France, [2]IGH Montpellier, Montpellier, France, [3]Cancer Research Center of Marseille, Marseille, France, [4]IPBS Toulouse, Toulouse, France, [5]CBI Toulouse, Toulouse, France, [6]IUCT Oncopole Toulouse, Toulouse, France, [7]CHU Rangeuil Toulouse, Toulouse, France.

Cytidine deaminase (CDA) converts cytidine and deoxycytidine into uridine and deoxyuridine within the pyrimidine salvage pathway for DNA and RNA synthesis. In this regard, loss of CDA provokes genomic instability in Bloom Syndrome, and CDA overexpression is associated with tumor resistance to chemotherapy with pyrimidine analogs. However, the precise role of CDA *per se* in cancer has been totally underexplored so far. Patient cohort analysis demonstrate that CDA is overexpressed in PDAC, a disease with no cure with increasing incidence, and associated with a worse prognosis in patient. Functional studies demonstrate that CDA is essential to PDAC cell proliferation and tumor growth. We found that CDA expression is associated with gene set enrichment in DNA replication signature, in both PDAC tumors and experimental models. Using enforced expression, genetic or pharmacologic targeting, we demonstrate that CDA promotes DNA replication, localizes to the replication fork and increases replication fork fitness. These effects are strictly dependent on CDA deaminase activity. Hence, we found that CDA controls replication stress as CDA expression is inversely correlated with the level of DNA breaks during S-phase, and that CDA targeting is associated with gene set enrichment in replication stress signature and CHK1 protein activation. Next, we demonstrate that CDA preserves genomic stability of PDAC cells, as CDA expression is inversely correlated with micronuclei formation and DNA damage transfer to daughter cells. We next explored PDAC cell lines from the Cancer Cell Lines Encyclopedia and found that CDA expression is associated with resistance to drug targeting DNA synthesis. Functional studies demonstrate that overexpressing CDA, and not CDA catalytically inactive mutant, protects PDAC cell lines from camptothecin, a topoisomerase inhibitor, while targeting CDA sensitizes PDAC cells to treatment by camptothecin and oxaliplatin, that forms platinum-DNA adducts. To gain further insights into the clinical potential of such finding, we targeted CDA expression in primary cells from patient with PDAC and found that CDA is essential to primary cell growth, and that CDA targeting sensitizes primary cells to treatment by oxaliplatin. Taken together, our results reveal for the first time that CDA controls DNA replication, replication stress level and genomic stability of PDAC cells, and that this new role of CDA is involved in tumor resistance to drugs inducing DNA damages. Thus, our work stems for new strategies based on CDA targeting to defeat PDAC resistance to treatment.

PO-044 Mechanobiological analysis of human patient pancreatic cancer tissues and the effect of cellular transmembrane mucins on glycocalyx-actomyosin mechanics. Andrew Massey. National Institutes of Health, Bethesda, MD.

Pancreatic cancer is one of the most lethal malignancies with a 5-year survival rate currently below 10%. Unfortunately, current diagnostic methods are unable to readily recognize early disease progression, and symptoms are commonly misdiagnosed. Therefore, determining novel biomarkers related to disease progression remains an important area of research. Transmembrane mucins, a major component of the cellular glycocalyx, normally play a protective role in epithelial tissues; they are also known to be overexpressed in various cancers, including pancreatic. In addition, mucins are known to increase aggressiveness, enhance drug resistance, and reduce survivability in cancers where they are upregulated. Although the biochemical effects of mucins are well understood, there is minimal research into how they affect cancer cells at a biophysical level. Recently, there has been great interest in examining the biophysical properties of cancer cells. The current consensus is that cancerous cells are softer than their normal counterparts, and that more metastatic cells become softer compared to more benign tumor cells.

Measuring these physical properties could potentially give clinicians a more rapid way to diagnose tumors, determine the course of disease progression, physically determine the effect of a biomolecule when its expression is altered, or determine the efficacy of various chemotherapeutics. In this study, we will first use atomic force microscopy-based nanomechanical mapping to measure the biophysical differences between normal cells, cancerous cells, and the extracellular matrix extracted from human patient tumor tissues and track the measured changes, both before and after chemo treatments. Our preliminary *in vitro* results suggest that 2D-adherent human cancerous pancreatic cells are indeed softer than their normal counterparts, in agreement with the literature. In addition, modulation of the glycocalyx architecture via hyaluronidase treatment leads to considerable changes in cellular stiffness in both normal and cancerous cells, implying a link between the glycocalyx and the underlying actomyosin skeleton. Future studies will examine the *in vitro* effects of specific transmembrane mucins. Using overexpression and knockdown transfection models, the impact on cellular mechanics, as well as structural changes in the glycocalyx and actomyosin cortex, will be analyzed in pancreatic cancer cells to determine how these mucins effect cellular mechanics and by extension regulate tumorigenesis and metastasis.

PO-045 Targeting HNF1A-dependent cell proliferation and stemness in PDAC using BET inhibitors. Bharani Muppavarapu, Ethan Abel, Melanie Mayberry. Roswell Park Comprehensive Cancer Center, Buffalo, NY.

Pancreatic ductal adenocarcinoma (PDAC) is a disease with poor prognosis, with an overall survival rate of only 10%. Standard of care involves chemotherapy which improves survival by only a few weeks. Several barriers to therapeutic efficacy impede increasing survival outcome, including intrinsic resistance, owing to presence of therapy-resistant pancreatic cancer stem-like cells (PCSCs). Therefore, developing therapeutics to target molecular networks that maintain PCSC populations is critical. Microarray analysis from our lab previously established that pancreas lineage transcription factor HNF1A and its targets are enriched in CD44+CD24+EPCAM+ human PCSCs and that genetic depletion of HNF1A ablates this population and importantly, diminished tumor growth in primary PDAC xenografts. However, there are no therapeutics to directly target HNF1A. In this project, we propose to indirectly ablate HNF1A and its network by targeting epigenetic readers, bromodomain and extra-terminal domain protein family (BET) that maintain expression of transcription factor networks. We hypothesize that BET inhibitors decrease stemness and tumor growth in a HNF1A-dependent manner. Studies in our lab established that BET inhibitors decrease colony-formation, tumorsphere formation and cell-cycle progression in PDAC cells, that is rescued by overexpressing HNF1A. These results indicate that BET inhibitors decrease stemness and proliferation in pancreatic cancer cells in a HNF1A-dependent manner. These results suggest that BET inhibitors target multiple HNF1A-dependent tumor-promoting programs, such as stemness and tumor growth which could impact utilization of these drugs in clinical settings. The ultimate goal of our project is to provide rationale for the use of BET inhibitors in clinical setting in pancreatic cancer.

PO-046 The effect of neoadjuvant therapy on immune profiling of pancreatic ductal adenocarcinoma: A prospective study of the PREOPANC-1 randomized controlled trial. Diba Latifi, Willem de Koning, Sai ping Lau, Frederiek Grevers, Coen van Dam, Casper H. J.

van Eijck, Dana A. M. Mustafa. Erasmus University Medical Center, Rotterdam, Netherlands.

The randomized phase III trial (PREOPANC-1) that was performed in 16 centers in the Netherlands compared the effects of preoperative chemoradiotherapy (Gemcitabine and 2.4 Gy radiation) versus immediate surgery for resectable and borderline resectable pancreatic cancer. The outcomes of the secondary endpoints and predefined subgroup analyses suggest an advantage of the neoadjuvant approach. The aim of the present study was to investigate the changes in the immune microenvironment and infiltration caused by the neoadjuvant treatment. To that aim, we collected formalin-fixed, paraffin-embedded pancreatic cancer samples from all centers that participated in the PREOPANC -1 trial. We performed targeted gene expression using the PanCancer Immune Profiling panel of NanoString. Comparing 50 samples of the patient who were subjected to neoadjuvant treatment to 46 treatment-naïve samples showed a distinct genetic profile induced by the neoadjuvant therapy. More than 250 immune-related genes were significantly differentially expressed between the two groups of samples. The results indicate that neoadjuvant therapy resets the innate immune activation in the tissue samples. A significantly higher infiltration of CD14+, CD33+, CSF1R+, and CD163+, MRC1+ cells were found in samples of the neoadjuvant arm. In contrast, B cells and various subtypes of T cells like CD8+ and FOXP3+ showed a significant decrease in the same samples. Pathway analysis revealed that the neoadjuvant treatment stimulated the expression of genes related to the complement activation, chemotaxis, and wound repair, while genes related to lymphocyte activation and adaptive immune responses were dominant in the treatment-naïve arm. In conclusion, this is the first comprehensive study to describe the immune-molecular changes as a result of neoadjuvant therapy in a randomized clinical trial. The results reveal the enrichment of the myeloid compartment following neoadjuvant therapy which was significantly associated with a survival benefit for the patients. Studying the personalized effect of neoadjuvant therapy will guide choosing the appropriate combined therapy for pancreatic cancer.

PO-047 Optimizing the efficacy of 5-FU as a chemotherapeutic agent in advanced pancreatic ductal adenocarcinoma (PDAC) using MIAPaCa-2 and PANC-1 cells. Nkafu Bechem Ndemazie, Andriana Inkoom, Xue Y. Zhu, Edward Agyare. Florida A&M University, Tallahassee, FL.

Purpose: Pancreatic ductal adenocarcinoma (PDAC) is the most aggressive and scourging soft tissue tumor worldwide. Its current incidence is 13 per 100,000 US population with a very low 5-years survival rate (5%) and estimated to be the second leading cause of cancer-related death by 2030. Most diagnoses are made at advanced disease states warranting combination chemotherapy. Fluorouracil (5-FU) is an effective drug in treating PDAC; however, rapid degradation and systemic instability remain a drawback to its efficacy. The objective of this study was to chemically modify 5-FU to 1,3 bistetrahydrofuran-2yl-5-FU (MFU) to prolong its systemic stability and enhance the anticancer activity of 5-FU. **Method:** 5-FU was chemically modified to MFU using tetrahydrofuran-2-yl acetate and 1,8-Diazabicyclo (5.4. 0)undec-7-ene (DBU) in Dimethyl Formamide (DMF). MFU was characterized using nuclear magnetic resonance (NMR) to determine new bond formation, mass spectrometer (MS) for molecular weight determination, high-performance liquid chromatography (HPLC) to determine percent purity, and micro-elemental analysis used to ascertain the presence of elemental composition and purity. Percent conversion of MFU to 5FU was determined using PDAC cells. In vitro anticancer

activity of MFU was tested using 2D and 3D MiaPaCa-2 and PANC-1 PDAC cultures and cell viability study performed using the alamar blue assay. Apoptotic and cell cycle studies were performed on MFU treated MiaPaCa-2 and PANC-1 cells using flow cytometry. **Results:** Molecular weight of synthesized MFU ($C_{12}H_{15}FN_2O_4$) was determined to be 270.02 using the Electrospray Ionization (ESI) (MS $(M+H)^+$ = 270.08). While percent purity of MFU was found to be greater than 99.6%, melting point was determined to be 115±2 ^oC and retention time (RT) was 1.85 minutes. For MFU conversion to 5-FU studies, 4.1% of 25µM and 7.6% of 50µM of MFU incubated MiaPaCa-2 cells was converted to 5-FU over a period of 24 hr showing an incremental production of 5-FU from MFU based on concentration. For in vitro study, half-maximal inhibitory concentration (IC_{50}) of 5-FU treated 2D MiaPaCa-2 culture was 3.4 ±1.1µM while that of MFU treated 2D MiaPaCa-2 culture was 2.1± 0.2 µM. The IC_{50} value of 5-FU treated 3D MiaPaCa-2 culture was found to be (8.5±1.2µM), while IC_{50} value for MFU treated 3D MiaPaCa-2 culture was (7.2±1.1µM). Put together, IC_{50} value for MFU for either 2D or 3D was significantly lower compared with IC_{50} value 5-FU (P=0.021 (MFU vs 5-FU) for 2D and P=0.04 (MFU vs 5-FU) for 3D). A similar trend was noted when PANC-1 cells were used. Apoptotic and cell cycle studies were similar to that of the 5-FU with most of the cells (54%) in late apoptosis after 48 h of treatment and 64% vs 33% of cells arrested at the G1 and S phase of the cell cycle respectively. **Conclusion:** The studies demonstrated that MFU may be an alternate approach to improve the delivery, systemic stability, and efficacy of 5-FU in the treatment of PDAC tumors.

PO-048 A novel chromatin remodeling domain of keratin 17 regulates transcription and promotes tumor aggression in pancreatic cancer. Chun-Hao Pan[1], Robert Tseng[1], Simon J. Hogg[2], Gabriella Baraks[1], Cindy V. Leiton[1], Lucia Roa-Peña[1], Natalia Marchenko[1], Kenneth R. Shroyer[1], Luisa F. Escobar-Hoyos[3]. [1]Stony Brook University, Stony Brook, NY, [2]Memorial Sloan Kettering Cancer Center, New York, NY, [3]Yale University, New Haven, CT.

Pancreatic ductal adenocarcinoma (PDAC) is characterized by two molecular subtypes, of which the basal-like subtype is associated with the worst survival and is highly resistant to first-line chemotherapy. We previously reported that keratin 17 (K17), a component of the molecular signature of the basal-like subtype, is a negative prognostic and predictive biomarker, whose overexpression results in chemoresistance and shortened patient survival. Here, we aimed to uncover the mechanistic role of K17 in driving cancer aggression and explore the therapeutic opportunities in K17-expressing PDAC. Beyond its role as a cytoskeletal protein, K17 can solubilize from the filamentous form and translocate into the nucleus, where it impacts multiple cellular properties. Previous reports suggest that nuclear K17 regulates cell-cycle progression and gene expression. However, the detailed mechanisms through which K17 modulates gene expression remains unexplored. Through domain-prediction analyses, we identified a novel chromatin-remodeling (SANT) domain in K17 that may interact with histones to regulate chromatin structure. The assay for transposase-accessible chromatin using sequencing (ATAC-seq) revealed that K17 positive cells had comparably accessible regions relative to K17 negative cells. However, the deletion of the K17 SANT domain (dSANT) decreased chromatin accessibility, suggesting that nuclear K17 impacts chromatin structure via the SANT domain. With RNA sequencing (RNA-seq) pathway enrichment analysis, we found that K17 positive cells had differential expressed genes that are associated with cell signaling, immune regulation, and metabolic pathways. More specifically, by comparing wild type K17 and dSANT K17-

expressing cells, we found that the SANT domain impacts a wide variety of immune regulatory pathways, many of which affect the tumor microenvironment. We are now performing chromatin immunoprecipitation sequencing (ChIP-seq) with acetylation of lysine 27 on histone H3 protein subunit (H3K27ac), to reveal differential bindings that are impacted by K17 and the SANT domain. Importantly, mice bearing orthotopic xenografts of PDACs with K17-SANT deletion showed survival comparable to those with K17 negative tumors and had significantly longer survival than those with wild-type K17. In summary, we identified a novel chromatin remodeling domain in K17 that could be explored as a target for the development of a biomarker-based treatment for PDAC.

PO-049 Inhibiting MNK kinases promotes macrophage immunosuppressive phenotype to limit anti-tumor immunity. Thao N. D. Pham[1], Christina Spaulding[1], Mario A. Shields[1], Mahmoud G. Khalafalla[1], Daniel R. Principe[2], David J. Bentrem[1], Hidayatullah G. Munshi[1]. [1]Feinberg School of Medicine, Northwestern University, Chicago, IL, [2]Medical Scientist Training Program, College of Medicine, University of Illinois at Chicago, Chicago, IL.

Background MAPK-interacting serine/threonine-protein kinase 1 and 2 (MNK1 and MNK2) are downstream effectors of the MEK/ERK and p38 MAPK pathways. Increased expression and activity of MNK kinases are linked to tumor growth and therapeutic resistance. Select MNK kinase inhibitors are currently being evaluated in clinical trials for different tumor types. Their immunomodulatory effects in tumors with low infiltrating $CD8^+$ T have not been clearly defined. **Methods:** *In vivo* efficacy of MNK kinase inhibitors (CGP57380 and eFT508), either as a single agent or in combination with anti-PD-1 or anti-CSF-1R antibody, was tested in syngeneic mouse models of pancreatic cancer. Tumor-associated macrophages (TAMs) and murine bone marrow-derived macrophages (BMDMs) were evaluated *in vitro* for modulation of their polarization by MNK kinase inhibitors and their suppression of co-cultured T cells. Markers of M1/M2 polarization were measured by qRT-PCR. The effects of MNK kinase inhibitors on the expression of select M2 markers were also evaluated in *ex vivo* slice cultures of human pancreatic tumors. **Results:** We first found an inverse relationship between MNK kinase activity and $CD8^+$ T cell abundance in human pancreatic tumors. In tumor-bearing mice, while pharmacological inhibition of MNK kinase activity increased $CD8^+$ T cell infiltration, the tumor-infiltrating $CD8^+$ T cells lacked effector function and failed to mount anti-tumor responses. Mechanistically, we showed that systemic inhibition of MNK kinases increased the expression of several anti-inflammatory genes in BMDMs and potentiated the ability of BMDMs and TAMs to suppress T cell proliferation. Reversal of T cell exhaustion either by an anti-PD-1 antibody or by TAM depletion with an anti-CSF-1R antibody enhanced the anti-tumor efficacy of MNK inhibitors and prolonged animal survival. Importantly, treating *ex vivo* human pancreatic cancer slice cultures with MNK inhibitors led to increased expression of known immunosuppressive markers in TAMs. **Conclusion:** Together, these findings provide new insights into the effects of MNK kinase inhibition on $CD8^+$ T cell infiltration and TAM function and identify combination regimens with MNK kinase inhibitors to achieve effective anti-tumor responses in pancreatic cancer patients whose tumors have a low number of functional $CD8^+$ T cells.

PO-050 Precision Promise (PrP): An adaptive, multi-arm registration trial in metastatic pancreatic ductal adenocarcinoma (PDAC). Vincent J. Picozzi[1], Anne-Marie Duliege[2], Anirban Maitra[3], Manuel Hidalgo[4], Andrew Eugene Hendifar[5], Gregory L. Beatty[6], Sudheer

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Background: Drug development in PDAC has been disappointing with an extremely low trial success rate despite considerable effort. PrP is a transformative, adaptive clinical trial platform that attempts to correct this by continuously and rapidly evaluating novel therapeutic options while maximizing the probability of patient (pt) randomization to an experimental treatment and nurturing enhanced cooperation among groups representing pt advocacy, pharmaceuticals, translational/clinical academia, and the FDA. This patient-centric study represents a fundamental shift in drug development for PDAC in the United States and aims to become the largest Phase 2/3 registrational study ever launched in this disease. **Methods:** PrP (NCT04229004) is a clinical trial platform sponsored by the Pancreatic Cancer Action Network (PanCAN) and funded solely through non-government sources. The protocol was finalized based on the FDA 2020 guidance document regarding "complex innovative designs" in registration trials https://www.fda.gov/media/130897/download. It utilizes adaptive randomization along with several trial design and Bayesian statistical innovations provided by Berry Consultants LLC. All pts undergo pre-and on-treatment biopsies with state-of-the-art genomic, transcriptomic, and immune analysis, along with collection of blood samples for research purposes throughout the study. Pts are managed using novel standardized supportive care techniques, and PrP contains 3 sub-protocols involving quality of life, sarcopenia and actigraphy. PrP was launched in 2020, and currently includes 20 US sites. Focused on both 1st and 2nd line treatment of metastatic PDAC, PrP uses an adaptive platform design with 30% of pts randomized between one of the 2 standard of care control arms (gemcitabine + nab-paclitaxel and mFOLFIRINOX) and 70% to experimental arms, currently either SM-88, a cancer metabolism-based agent (Tyme Inc); or Pamrevlumab, an antibody inhibiting the activity of the connective tissue growth factor (Fibrogen Inc.) The study is ongoing with >100 pts enrolled to date. The Data and Safety Monitoring Committee regularly reviews the data and continues to recommend that the trial proceeds as planned. New study arms will be added after review by an Arm Selection Committee that assesses the validity of the treatment target and the adequacy of the preexisting pre-clinical and clinical data. An additional experimental arm is anticipated in 2021. **Conclusion**: Compared to traditional trial designs, PrP offers several advantages: multiple investigational treatments can be evaluated in parallel over time; only ~ 175 pts per experimental arm required to initiate a regulatory registration; and increased learning from every patient during the trial, altogether resulting in both time saving and a 30-50% cost saving. In effect, PrP has created an entirely new "learning community" and can substantially accelerate drug development for PDAC.

PO-051 PANOVA-3: A phase III study of tumor treating fields with nab-paclitaxel and gemcitabine for front-line treatment of locally advanced pancreatic adenocarcinoma.

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Tumor Treating Fields (TTFields) are a non-invasive, loco-regional antimitotic treatment modality, approved for the treatment of glioblastoma and malignant pleural mesothelioma. TTFields at a specific frequency (150-200 kHz) are delivered via arrays placed on the skin surrounding the tumor site. TTFields predominantly act by disrupting the formation of the mitotic spindle during metaphase. TTFields were effective in multiple preclinical models of pancreatic cancer. The Phase 2 PANOVA study, the first trial testing TTFields in pancreatic cancer patients, demonstrated the safety and preliminary efficacy of TTFields when combined with nab-paclitaxel and gemcitabine in both metastatic and locally advanced pancreatic adenocarcinoma (LAPC). The Phase 3 PANOVA-3 trial (NCT03377491) is designed to test the efficacy and safety of adding TTFields to nab-paclitaxel and gemcitabine combination in LAPC. Patients (N = 556) with unresectable, LAPC (per NCCN guidelines) will be enrolled in this prospective, randomized trial. Patients should have an ECOG score of 0-2 and no prior progression or treatment. Patients will be stratified based on their performance status and geographical region, and will be randomized 1:1 to TTFields plus nab-paclitaxel and gemcitabine or to nab-paclitaxel and gemcitabine alone. Chemotherapy will be administered at standard dose of nab-paclitaxel (125 mg/m2) and gemcitabine (1000 mg/m2) on day 1, 8, and 15 of a 28 day cycle. The protocol has recently been amended to incorporate the use of a smaller, lighter (weight reduced from 6 to 2.7 lbs.) TTFields device. TTFields (150 kHz) will be delivered at least 18 hours/day until local disease progression per RECIST Criteria V1.1. Follow up will be performed q4w, including a CT scan of the chest and abdomen q8w. Following local disease progression, patients will be followed monthly for survival. Overall survival will be the primary endpoint. Progression-free survival, objective response rate, rate of resectability, quality of life, and toxicity will all be secondary endpoints. Sample size was calculated using a log-rank test comparing time to event in patients treated with TTFields plus chemotherapy with control patients on chemotherapy alone. PANOVA-3 is designed to detect a hazard ratio 0.75 in overall survival. Type I error is set to 0.05 (two-sided) and power to 80%.

PO-052 A pilot study of miRNA expression profile in surgically resected pancreatic ductal adenocarcinoma: Initial report from a bi-institutional cohort. Luca Pompella[1*], Michela Falco[2*], Carlo Caputo[2*], Anna Grimaldi[2], Giuseppe Tirino[1], Severo Campione[3], Francesca Sparano[1], Maria Lucia Iacovino[1], Chiara Carmen Miceli[1], Carlo Molino[4], Marco Montella[5], Renato Franco[5], Gennaro Galizia[6], Giovanni Conzo[7], Vincenzo Napolitano[7], Annamaria Auricchio[6], Francesca Cardella[6], Fortunato Ciardiello[1], Michele Caraglia[2], Angela Lombardi[2], Gabriella Misso[2*] and Ferdinando De Vita[1*]. [1]Department of Precision Medicine, Division of Medical Oncology, University of Campania "L. Vanvitelli", Aversa, Italy, [2]Department of Precision Medicine, Division of Molecular Pathology, University of Campania "Luigi Vanvitelli", Naples, Italy, [3]Department of Precision Medicine, Division of Medical Oncology, University of Campania "L. Vanvitelli", Naples, Italy, [4]Division of Surgical Pathology, AORN "Antonio Cardarelli", Naples, Italy, [5]Division of General Surgery 1, AORN "Antonio Cardarelli", Naples, Italy, [6]Division of Surgical Pathology, University of Campania "Luigi Vanvitelli", Naples, Italy, [7]Department of Surgical Sciences, University of Campania "Luigi Vanvitelli", Naples, Italy, [8]Department of Translational Medical Sciences, University of Campania "Luigi Vanvitelli", Naples, Italy, [9]Department of Precision Medicine, Division of Molecular Pathology, University of Campania "L. Vanvitelli", Naples, Italy. * These authors

contributed equally to this work.

Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal human malignancies: novel therapeutic approaches beyond conventional chemotherapy are still lacking and prognosis remains poor, even for resectable patients (pts). Furthermore, there is an almost complete absence of validated predictive factors. Consequently, robust biomarkers for the early diagnosis and the prognostic stratification are urgently needed in clinical practice, especially in the context of neoadjuvant and adjuvant settings. In the last years, evidence revealed the crucial role of miRNAs in cancer initiation and progression, as well as in the chemo-resistance mechanisms, suggesting their use as clinical biomarkers. **Material and methods**: In this pilot study, we performed a microarray analysis to characterize global miRNA expression profile from surgical tissue samples collected from 20 resected PDAC pts pooled into 4 groups according to different clinico-pathological features: nodal metastases (N+/N-) and tumor grading (G2/G3). **Results**: According to expression patterns, we identified, among 384 miRNAs, a significant different modulation for 11 miRNAs associated to G2 vs G3 and for 7 miRNAs in N+ vs N- disease, suggesting a possible specific signature reflecting histological grade and nodal metastasis occurrence, respectively. We focused on 2 up-regulated (miR-138-5p and miR-518-3p) and 3 down-regulated (miR-215-5p, miR-519a-3p and miR-576-5p) miRNAs in N+ pts, and on 3 up-regulated (miR-1-3p, miR-31-5p and miR-205-5p) in G3 pts, in order to verify their possible implication in the molecular changes behind tumor differentiation and spread, as well as their potential use for prognostic and therapeutic purpose. A bio-informatic analysis was also performed, using different in silico tools, to study both high affinity miRNA targets and cross-regulated pathways among the upand down-regulated miRNAs. The results identified several associated targets involved in multiple signaling pathways commonly dysregulated in cancer. Finally, BRCA1/2 and RB1 miRNAs-mediated-modulation is actually ongoing, considering the pivotal role of these genes in some PDAC pts. **Conclusion**: These preliminary data provide a strong rationale to further investigate miRNAs expression in larger cohorts of PDAC pts, possibly integrating validated tissue miRNAs data with circulating miRNAs, in order to identify strong (and easily accessible) potential biomarker(s) with prognostic and/or predictive significance.

PO-054 A phase II trial of the super-enhancer inhibitor Minnelide in advanced refractory adenosquamous carcinoma of the pancreas (ASCP). Nebojsa Skorupan[1], Mehwish I. Ahmad[1], Seth M. Steinberg[1], Jane B. Trepel[1], Derek Cridebring[2], Haiyong Han[2], Daniel D. Von Hoff[2], Christine Alewine[1]. [1]CCR, Bethesda, MD, [2]Translational Genomics Research Institute, Phoenix, AZ.

Background: Adenosquamous carcinoma of the pancreas (ASCP) is a very rare and highly aggressive variant of pancreatic ductal adenocarcinoma (PDA), accounting for 0.5-4% of all pancreatic cancer cases in the US. Current data indicates that epigenetic changes and Myc overexpression lead to squamous transdifferentiation of pancreatic tumor cells and development of ASCP. Minnelide is an oral anti-super-enhancer drug that inhibits Myc expression in preclinical models of ASCP. Oral Minnelide has been previously tested in the clinic where a Phase I study determined a recommended phase 2 dose of 2 mg/day. Major toxicities included neutropenia and sepsis. We hypothesize that Minnelide will be safe and effective in patients with advanced ASCP. **Methods**: This Phase II open label, single arm study being conducted at the

NIH Clinical Center in Bethesda, MD (NCT04896073) will enroll up to 25 evaluable participants with advanced ASCP that has been previously treated. Eligibility criteria include: histologically confirmed ASCP (≥30% malignant squamous component), presence of metastatic, recurrent, or locally advanced unresectable disease, and progression or intolerance ≥1 prior systemic treatment for advanced disease. All participants will self-administer Minnelide at 2mg PO daily on days 1-21 of each 28-day cycle, for up to 12 cycles in the absence of disease progression or unacceptable toxicity. Restaging scans will be performed every 8 weeks. Optional biopsies will be requested at baseline, mid-cycle 1 and at time of progression. The primary objective is to determine the antitumor activity of Minnelide by evaluating the disease control rate. Secondary objectives are determination of drug safety in this patient population, progression free survival, and overall survival. Exploratory objectives include assessment of Minnelide effect on tumor cell epigenetics, Myc expression and immune cell populations using on-treatment tumor biopsy tissues. Changes in circulating tumor DNA and circulating immune cell populations upon treatment will also be evaluated. A Simon optimal two-stage phase II trial design will be used. If 3 of the first 12 patients experience disease control, then enrollment will continue up to 25 evaluable participants. If ≥8 of 25 participants experience disease control this would rule out an unacceptably low clinical benefit of 20% in favor of 40% ($\alpha = 0.1$, $\beta = 0.2$). This trial opened for accrual in July 2021.

PO-055 Phase II clinical trial of subtype directed neoadjuvant therapy in patients with localized pancreatic cancer. Susan Tsai[1], Erkut Borazanci[2], Margaret Gulley[3], Naim Rashid[3], Jason Merker[3], Abdul H. Khan[1], Phillip Chisholm[1], Bryan Hunt[1], Tamara Giorgadze[1], William Hall[1], Mandana Kamgar[1], Douglas B. Evans[1], Jen Jen Yeh[3]. [1]Medical College of Wisconsin, Milwaukee, WI, [2]Honor Health Medical Group, Scottsdale, AZ, [3]University of North Carolina, Chapel Hill, NC.

Background: Preoperative (neoadjuvant) therapy has become the preferred treatment sequencing strategy for patients with localized pancreatic cancer. During neoadjuvant therapy, approximately 30% of patients will experience metastatic disease progression while on treatment. Therefore, tools to aid clinicians to select efficacious first-line chemotherapeutic regimens is a critical unmet need. The most common neoadjuvant chemotherapy regimens used are 5-fluorouracil/irinotecan/oxaliplatin (mFOLFIRINOX) and gemcitabine/nab-paclitaxel (GnP). There is growing data to suggest an association of pancreatic cancer subtype (classical versus basal-like) with treatment response to therapy. Recently, the translation of tumor subtyping to the clinic has been successfully achieved using the Purity Independent Subtyping of Tumors (PurIST) single sample classifier. We aim to assess the clinical response to pancreatic cancer subtype-directed therapy in patients with localized pancreatic cancer. **Methods:** This is a phase II, multicenter, single-arm clinical trial for previously untreated patients with localized (resectable or borderline resectable) pancreatic cancer. Patients will undergo endoscopic ultrasound guided biopsy of the primary tumor and PurIST classifier to determine classical versus basal-like subtype. Patients with classical subtype will be assigned to mFOLFIRINOX and patients with basal-like tumors will be assigned to GnP. Following two months of therapy, patients will be restaged with a computed tomography scan, carbohydrate antigen (CA19-9) levels, performance status assessment, and a repeat endoscopic ultrasound guided biopsy for research. The primary endpoint is composite clinical response as measured by radiographic response, CA19-9 decline, and performance status following two months of treatment.

Correlative endpoints include blood-based biomarkers for association with clinical response and stroma-specific response to therapy. The study has enrolled 4 of the anticipated 41 patients at the time of submission. Clinical Trial information: NCT 04683315.

PO-056 Insulin receptor signaling in pancreatic acinar cells contributes to pancreatic cancer development. Anni M. Y. Zhang, Jenny C. C. Yang, Twan J. J. de Winter, David F. Schaeffer, Janel L. Kopp, James D. Johnson. The University of British Columbia, Vancouver, BC, Canada.

Hyperinsulinemia is a cardinal feature shared by both obesity and type 2 diabetes, and is independently associated with increased risk of pancreatic ductal adenocarcinoma (PDAC). We previously showed a ~50% reduction in pancreatic intraepithelial neoplasia (PanIN) pre-cancerous lesions in mice with genetically reduced insulin production. Our single-cell transcriptomic data suggested that many pancreatic cell types could mediate the effects of local hyperinsulinemia on PanIN development. In pancreatic acinar cells from mice with reduced insulin, we found alterations in the PI3K/AKT/mTOR and MAPK/ERK pathways known to be involved in tumorigenesis. Here, we examined whether hyperinsulinemia contributes to PDAC development directly through insulin receptor signaling in *Kras*^{G12D} expressing pancreatic acinar cells. To test this hypothesis, we generated *Ptf1a*^{CreER};LSL-*Kras*^{G12D};nTnG mice with an *Insr*$^{wt/wt}$ (PK-*Insr*$^{wt/wt}$), *Insr*$^{wt/fl}$ (PK-*Insr*$^{wt/fl}$), or *Insr*$^{fl/fl}$ (PK-*Insr*$^{fl/fl}$) genotype to reduce insulin receptor signaling by 0%, 50%, or 100% in acinar cells, in both males and females. We fed the mice with high-fat diet (HFD) to induce systemic hyperinsulinemia and tracked body weight, fasting glucose and fasting insulin levels routinely. We euthanized the mice when they were 10 months old and performed blinded histopathological analysis and immunohistochemistry staining of the pancreatic sections to assess the PanIN formation. Loss of insulin receptors from acinar cells did not significantly influence body weight, fasting glucose or fasting insulin levels. Alcian blue staining of mucins contained within low-grade PanINs showed that there was a significant reduction in these pre-cancerous lesions in PK-*Insr*$^{wt/fl}$ and PK-*Insr*$^{fl/fl}$ female mice compared to PK-*Insr*$^{wt/wt}$ mice and the difference was *Insr* gene dosage-dependent. By performing immunohistochemical staining of CK19, marking duct and duct-like cells, we found there was a significant reduction of CK19^{+} area in PK-*Insr*$^{wt/fl}$ and PK-*Insr*$^{fl/fl}$ mice compared to PK-*Insr*$^{wt/wt}$ mice, and the reduction was *Insr* gene dosage-dependent. Finally, we found a significant increase in retention of normal acinar cells in PK-*Insr*$^{fl/fl}$ mice compared to PK-*Insr*$^{wt/wt}$ mice, which indicates the mice losing *Insr* had more wild-type like pancreas. Collectively, these data strongly suggest that insulin receptor signaling in acinar cells is important for the metaplasia formation, but do not exclude a role of Insr on other local or distant cell types. Prophylactic approaches targeting insulin receptor signaling pathways, or hyperinsulinemia itself, may be beneficial in preventing pancreatic cancer.

PO-057 Targeting ErbB2 degradation via the ubiquitin–proteasome pathway to inhibit the metastasis of pancreatic cancer. Bo Zhang, Fei Teng, Nengming Lin. Hangzhou First People's Hospital, Hangzhou, China.

Objective: Pancreatic cancer is currently one of the most lethal human cancers and continues to be a major unsolved health problem in the 21st century. Since pancreatic cancer has a high metastatic potential, chemotherapy is inevitable for most pancreatic

cancer patients, whereas the clinical responses to anticancer agents are not satisfied. In this work, we designed and synthesized a novel compound, termed as 6-MPA, and found that 6-MPA could improve the proteasomal degradation of ErbB2 pancreatic cancer cells. **Methods:** The inhibitory effects of 6-MPA on migration and invasion was measured by scratch experiment, wound healing assay and transwell experiment. Clone formation and CCK-8 assay was used to determine the cytotoxicity of 6-MPA. Real-time PCR and western blotting was used to determine the expression of indicated genes or proteins respectively. Immunoprecipitation was used to characterize protein–protein interactions. **Results:** 4, 8, and 16 μM of 6-MPA exhibited an inhibitory effects on the migration of SW1990 cells and the inhibition rates reached 40.2±10.1 %, 58.2±11.2 %, and 65.2±9.9 % in vitro, respectively. RNA-seq showed that 6-MPA treatment manipulated ErbB2 level and its downstream PI3K/AKT/mTOR signaling pathways, which was verified by western blotting assay. Treatment with 6-MPA could enhance the interaction between ErbB2 and ubiquitin, and thus promote the proteasomal degradation of ErbB2. **Conclusion** In this study, we obtained a novel small molecule 6-MPA, which could specifically degrade ErbB2 through the activation of protein ubiquitinination. And we found that 6-MPA possessed potent inhibitory effects on migration and invasion of pancreatic cancer cells. Therefore, our work illustrated a novel structure skeleton that could be used to target ErbB2, and potentially suppressed the metastasis of pancreatic cancer.

Preclinical Models

PO-058 Anti-cancer activity of NTAX-44 (bioprocessed arsenic trioxide) on pancreatic cancer cell line. Yogesh Bendale[1], Padma Shastri[2], Radha Poojari[3], Nandinee Khot[2], Surendra Nagare[2], Avinash Kadam[2]. [1]Rasayu Cancer Clinic, Pune, India, [2]Rasayani Biologics Pvt. Ltd., Pune, India, [3]Innovation Centre, Tata Chemicals Ltd., Pune, India.

Purpose: Pancreatic Cancer remains to be highly lethal malignancy mainly because of a high incidence of drug resistance. Because of this, there is a great need for new therapies which can improve overall therapeutic outcomes in pancreatic cancer patients. Arsenic trioxide is an approved drug for the treatment of acute promyelocytic leukemia (APML) and is in the experimental stage for use in different malignancies. Earlier *in-vitro* studies on human pancreatic cell lines have shown that arsenic trioxide has potent anti-proliferative effects with induction of apoptosis. NTAX-44 is a novel orally administrable Arsenic trioxide that is derived using green chemistry based patented technology. This compound is bioavailable when administered orally, and it has demonstrated good safety and tolerability in bioavailability clinical trials. In the present study, we conducted preclinical studies to evaluate the anti-proliferative effect and inhibition of programmed cell death ligand-1 (PD-L1) expression activity by NTAX-44. **Method:** The effect of NTAX-44 was assessed on the viability of MIA-Pa-Ca-2 cell lines grown as monolayers by MTS assay. We further checked the expression of PD-L1 in MIA-Pa-Ca-2 cell line treated with NTAX-44 by flow cytometry. PD-L1, an immune checkpoint regulator, has been speculated to play a significant role in suppressing the *immune s*ystem. It also helps tumor cells evade anti-tumor *immunity. The expression of PD-L1 was induced by IFN-gamma stimulation.* The anti-metastatic capacity of NTAX-44 was evaluated by *in ovo* chick embryo yolk sac membrane (YSM) angiogenesis assay. *The* cytocompatibility testing was done by testing the effect of NTAX-44 on the viability of normal human peripheral blood mononuclear cells (PBMC) by MTS assay. **Results**: NTAX-44 effectively inhibited the viability of MIA-Pa-

Ca (IC50 4.63 +/- 1.16µg/ml for 48 hr and 0.50 +/- 0.63 for 72 hr of treatment); demonstrating the anti-proliferative activity of NTAX-44 against MIA-Pa-Ca-2 cell line. Furthermore, NTAX-44 at 25µg/ml concentration significantly inhibited IFN-gamma-induced PD-L1 expression in the MIA-Pa-Ca-2 cell line. Evaluation of the anti-metastatic potential of NTAX-44 exhibited *around 40% inhibition in the number of quaternary blood vessels was observed on the treatment of YSM with NTAX-44 compared to vehicle control. It is noteworthy that the anti-angiogenic activity was comparable to that of Bevacizumab (Avastin®).* The cytocompatibility testing revealed that it was well tolerated at a concentration up to 200µg/ml in PBMC cells.
Conclusions: The preclinical data presented in this study suggests the potential of NTAX-44 to inhibit proliferation and, PD-L1 expression in MIA-Pa-Ca-2 cells and prevent angiogenesis in YSM. These properties along, with their good tolerability as indicated by cytocompatibility testing, are indicative of the potential role of NTAX-44 as an oral chemotherapeutic agent in the treatment of Pancreatic cancer. Though the preclinical studies performed on NTAX-44 are very promising but further clinical evaluation is warranted.

PO-059 Epithelial/mesenchymal identity dictates pancreatic cancer cell metastasis.
Julienne L. Carstens[1], Sujuan Yang[1], Pedro Correa de Sampaio[1], Xiaofeng Zheng[1], Souptik Barua[2], Kathleen M. McAndrews[1], Arvind Rao[3], Jared K. Burks[1], Andrew D. Rhim[1], Raghu Kalluri[1]. [1]MD Anderson Cancer Center, Houston, TX, [2]Rice University, Houston, TX, [3]University of Michigan, Ann Arbor, MI.

Metastatic pancreatic adenocarcinoma (PDAC) is the dominant clinical presentation and highly treatment-resistant. However, not all metastasis is equal with metastatic disease in the lung having improved outcomes over the liver. These clinical differences suggest a therapeutic opportunity and urge analysis of the molecular underpinnings of PDAC metastasis. The acquisition of mesenchymal features by epithelial cancer cells is commonly associated with solid tumor metastasis and has been linked to the pancreatic cancer basal subtype and its association with treatment resistance and poorer outcomes, but its impact on pancreatic cancer metastasis needs further understanding. We explored the impact on metastasis of stabilized epithelial, partial-mesenchymal and mesenchymal cancer cells by generating several genetic mouse models based on the lineage-traced KPC mouse model (**K**ras$^{LSL\text{-}G12D}$;**p**53^{R172H}; or **p**53^{LSL}; PDX1-**C**re;EYFP^{LSL}). Using single-cell RNA-sequencing we confirmed the KPC mouse model recapitulates the spectrum of epithelial-mesenchymal phenotypes observed in patients and can be genetically engineered to stabilize specific phenotypes. The stabilization of epithelial phenotypes through the homozygous loss of the mesenchymal-driving transcription factors Snail and Twist (Snai1$^{F/F}$;Twist1$^{F/F}$) had no impact on primary tumor progression but increased liver colonization. This increase in liver colonization was supported by a second epithelial-stabilized mouse model based on the loss of Zeb1 (Zeb1$^{F/F}$). The stabilization of mesenchymal features through the heterozygous or homozygous loss of the epithelial adherin junction E-cadherin (Cdh1$^{F/+}$ or Cdh1$^{F/F}$) promoted lung metastasis. Interestingly, epithelial plasticity was still required for efficient lung colonization, but not rare liver metastasis. Additionally, mesenchymal gene expression correlated with an improved patient survival as well as metastatic localization, supporting the clinical observations of improved survival in lung metastasis. Using gene expression analysis of sorted bulk cancer cells and single-cells, migration assays, and multiplexed-immunohistochemistry, we observed that the epithelial/mesenchymal status of the cancer cells dictated different mechanisms for motility and interaction with the immune system.

Mesenchymal cancer cells migrate as single-cells and attract fewer T-cells where epithelial cancer cells migrate collectively and have increased immune regulation gene expression. These data suggest the epithelial/mesenchymal status of cancers cells dictate the where and how of metastatic disease and could inform therapeutic interventions.

PO-060 N-terminal RHAMM cooperates with dysfunctional p53 to accelerate the progression of pancreatic cancer. Anthony Lin[1], Jennifer Feng[1], Xiang Chen[1], Dunrui Wang[2], Megan Wong[1], George Zhang[1], Joseph Na[1], Tiantian Zhang[1], Zhengming Chen[1], Yao-Tseng Chen[1], Yi-Chieh Nancy Du[1]. [1]Weill Cornell Medicine, New York, NY, [2]National Institutes of Health, Bethesda, MD.

Pancreatic cancer has the lowest survival rate in all types of cancer. Pancreatic cancer patients are often diagnosed at advanced stages. A better therapeutic development for this devastating disease is urgently needed. Receptor for hyaluronan-mediated motility (RHAMM), not expressed in adult normal pancreas, has been suggested as a prognostic factor and a potential therapeutic target for pancreatic ductal adenocarcinoma (PDAC) and pancreatic neuroendocrine tumor (PNET). In this study, we initially sought to determine whether genetic deletion of *RHAMM* would slow down pancreatic cancer progression using *Rhamm*$^{-/-}$ mice. However, we found that *Rhamm*$^{-/-}$ mice expressed a N-terminal RHAMM protein. While N-terminal RHAMM did not enable malignant progression of pancreatic intraepithelial neoplasia in *p48-Cre*; *LSL-KRAS*^{G12D} mice, it accelerated the formation of invasive PDAC and shortened the survival of *p48-Cre*; *LSL-KRAS*^{G12D} mice with heterozygous p53 knockout. *Kras*^{G12D} PDAC mice with homozygous p53 knockout mice died around 10 weeks, and the effect of N-terminal RHAMM was not apparent in these mice with short life span. In addition, N-terminal RHAMM shortened the survival of PNET-bearing *RIP-Tag* mice, which had inactivated p53. In our analysis of TCGA dataset, pancreatic cancer patients with mutant *TP53* or loss of one copy of *TP53* had higher *RHAMM* expression, which, combined, predicted worse outcomes. Taken together, by collaborating with dysfunctional p53, N-terminal RHAMM that lacks the centrosome targeting domain and degrons for interaction with the Anaphase-Promoting Complex (APC) accelerated pancreatic cancer progression.

PO-061 Myc drives phenotypic heterogeneity, metastasis, and therapy resistance in pancreatic ductal adenocarcinoma. Isabel A. English[1], Patrick J. Worth[1], Amy T. Farrell[1], Brittany L. Allen-Petersen[2], Vidhi Shah[1], Courtney Betts[1], Xiaoyan Wang[1], Colin J. Daniel[1], Mary C. Thoma[1], Lisa M. Coussens[1], Ellen M. Langer[1], Rosalie C. Sears[1]. [1]Oregon Health & Science University, Portland, OR, [2]Purdue University, West Lafayette, IN.

Pancreatic ductal adenocarcinoma (PDAC) ranks among the top three most aggressive cancers in the United States and is projected to increase in incidence over the next few years. Standard of care treatment for PDAC consists of a cocktail of harsh chemotherapies, which have improved overall survival by only a few percentage points – to a 5-year survival rate of 10%. One commonly deregulated pathway in PDAC is c-MYC (MYC), a potent transcription factor. MYC plays an important role in tumor progression and its deregulation has been correlated with tumor aggressiveness and therapeutic resistance in PDAC and other cancers. Recently, oncogenic MYC expression has been shown to regulate elements of the tumor microenvironment (TME) in mouse models of multiple cancers. In PDAC, MYC's expression has been linked to a desmoplastic

immune suppressive TME, yet the specific mechanism has yet to be described. Here, in order to better model the disease and to interrogate questions of *how* MYC regulates the tumor immune and stromal microenvironment, we have generated a novel genetically engineered mouse model (GEMM) of PDAC. Our model (KMC^{ERT2}) has inducible Cre-driven expression of both mutant Kras and low deregulated Myc in the pancreas. We have found that deregulated MYC cooperates with $KRAS^{G12D}$ in the adult pancreas to drive PDAC in our inducible KMC^{ERT2} mouse model and that our model recapitulates inter- and intra-tumoral heterogeneity seen within clinical PDAC populations as well as consistent metastasis to liver in both spontaneous and orthotopic transplant settings. Currently, a majority of murine studies of PDAC are performed using an embryonic $Kras^{G12D}$- and p53 loss/mutant-driven PDAC model (KPC). In contrast to the KPC model, our inducible KMC^{ERT2} model of PDAC displays genetic changes, such as CDKN2A and SMAD4 loss, comparable to human disease. Interestingly, multiplexed immunohistochemistry analysis of immune cell composition of spontaneous KMC^{ERT2} tumors compared to the commonly used KPC shows an increased density of antigen presenting cells (APCs) within MYC-driven tumors. Human PDAC is often resistant to standard of care therapies such as gemcitabine and FOLFIRINOX. Orthotopic therapeutic studies using our KMC^{ERT2} cell lines demonstrate a similar resistance to these therapies. To further understand the mechanisms underlying our observed phenotypes, we have conducted RNAseq and DNA sequencing on both microdissected autochthonous tumor specimens and KMC^{ERT2} tumor-derived cell lines. Together, this work investigates the role of deregulated MYC expression in metastatic behavior, immune phenotypes, and therapeutic response in murine PDAC. It also provides both spontaneous and orthotopic mouse models of PDAC that recapitulate the heterogeneous and highly metastatic nature of the human disease, allowing for important therapeutic testing opportunities.

PO-062 EUS-guided biopsy of pancreatic mass lesions for the development of patient-derived organoids in Puerto Rico. Andrea S. Flores Pérez[1], Janet Mendez Vega[1], Ana M. Reyes Ramos[1], Carlos Micames[2], Madeline Torres-Lugo[1], Maribella Domenech[1]. [1]University of Puerto Rico - Mayagüez, Mayagüez, Puerto Rico, [2]Hospital Bella Vista, Mayagüez, Puerto Rico.

Pancreatic cancer is an aggressive cancer that is diagnosed early in only 10% of cases and has a 5-year survival rate of less than 9%. The process in determining the optimal treatment for each patient is both long and tedious – and current strategies do not enable therapy optimization at the individual level. The incidence rate for pancreatic cancer in Puerto Rico between 2013 and 2017 was 8.2%, which is lower than the rate reported for non-Hispanic whites and blacks (16% and 12.9%, respectively). The causes for such health disparity are unknown but it is very likely associated with changes at the genomic and tissue microenvironment level. To identify patient and sub-population specific markers, patient-derived models need to be integrated into studies of drug screening and efficacy. Patient-derived organoids (PDO) are becoming a potential model for diagnosis and the study of disease progression due to their ability to recreate the pathology of the tumor in patients. Although several PDO studies have highlighted their potential for therapeutic applications, the ability to successfully culture and propagate these using standard methods has not been established in samples collected from the Hispanic population. We assessed the feasibility of establishing PDOs from tissue specimens of a pancreatic mass lesion in Puerto Rican patients using standard methods for pancreatic organoids. A total of 10 samples were collected from endoscopic ultrasound (EUS) fine-needle biopsies to examine the

pathological features of the tissue and the retention of the parental tumor characteristics in culture. All tissue specimens were cultured in Matrigel domes within 24 hours of extraction and organoid establishment was monitored for 7-8 days. All samples were positive for pancreatic adenocarcinoma but only 50% of them were successfully cultured in Matrigel. High ATP levels were observed in tumor organoids after one week in culture which is indicative of a high cell viability. Examination of pathological markers indicate that all samples were negative for epidermal growth factor (EGFR), 5/6 were positive for smooth muscle actin (SMA), 4/6 were positive for Vimentin, and 1/6 were positive for CA 19.9. Absence of EGFR was unexpected as this marker is highly expressed and abundant in 40-70% of pancreatic cancers. Samples with high levels of SMA, a fibroblast marker, resulted in a higher organoid yield than samples with no SMA present. Overall, the data suggests that the presence of fibroblasts is supportive of organoid establishment in culture and that standard culture methods need to be optimized to increase organoid yields. Future work will seek to increase the sample size to test alternative propagation methods and examine drug sensitivity in patient-derived pancreas organoids of the Puerto Rican population.

PO-063 Functional interrogation of immune escape in neoantigen-expressing pancreatic cancer identifies a critical role for the CD155/TIGIT axis. William Freed-Pastor, Laurens Lambert, Zackery Ely, Nimisha Pattada, Arjun Bhutkar, Alex Jaeger, George Eng, Kim Mercer, William Hwang, Tyler Jacks. MIT, Cambridge, MA.

The CD155/TIGIT axis can be co-opted during immune evasion in chronic viral infections and cancer. While insights regarding mechanisms of immune escape have fueled tremendous clinical successes in a broad range of tumor types**,** microsatellite-stable pancreatic adenocarcinoma (PDAC), which represents greater than 98% of patients, is largely refractory to available immune checkpoint blockade**.** The recent recognition that a subset of pancreas cancer harbors potential neoantigens has intensified interest in defining the molecular and cellular mechanisms of immune evasion in PDAC to guide effective therapeutic strategies that leverage the adaptive immune system in this disease. However, difficulty in precisely defining the tumor-reactive T cell compartment has hampered prior efforts to delineate the full spectrum of mechanisms by which PDAC evades immune eradication. We recently developed multiple organoid-based and autochthonous preclinical models to delineate the molecular and cellular mechanisms of immune evasion in this subset of patients. Specifically, we utilized a high affinity MHC class I-restricted antigen ($OVA_{257–264}$) or recently described missense-derived MHC class I-restricted neoantigens (mutations in the laminin 4 alpha subunit or in alpha-1,3-glucosyltransferase). While a subset of animals either successfully clear these tumors, or are arrested in a state of immune-mediated tumor dormancy, all three neoantigen models lead to a significant subset of tumors that acquire the ability to evade immune clearance. In these preclinical models, we demonstrate that intratumoral neoantigen-specific $CD8^+$ T cells adopt multiple states of dysfunction, typified by T cell exhaustion (best marked by $TIGIT^+PD1^+$ co-positivity). Global profiling of the tumor-immune microenvironment in neoantigen-expressing murine PDAC compared to non-neoantigen-expressing PDAC identified an expected increase in $CD8^+$ T cells, and specifically in neoantigen-specific $CD8^+$ T cells, but also identified additional changes within the broader leukocyte contexture. Using flow cytometric and single-cell transcriptomic analyses, we demonstrate that human PDAC TILs express similar dysfunctional programs and are enriched in $TIGIT^+PD1^+$ TILs. Furthermore, we show that CD155, the high affinity TIGIT ligand, is highly

expressed on the surface of murine and human PDAC tumor cells. Using genetic (CRISPR-activation) and/or pharmacologic modulation, we functionally interrogate the CD155/TIGIT axis and demonstrate that increased signaling through CD155 and/or TIGIT is sufficient to promote immune evasion in neoantigen-expressing PDAC. Lastly, we identified a combination immunotherapy (TIGIT/PD-1 co-blockade plus CD40 agonism) that elicits profound anti-tumor responses in these preclinical models. Using a suite of high-resolution analyses, we are dissecting the mechanisms of effectiveness and resistance to this combination immunotherapy, which is set to enter clinical trials in pancreatic cancer later this year.

PO-064 ONC212 stimulates cytotoxic T-cell killing, increases tumor-immune cell interactions, and promotes tumor regression in combination with TLY012 in a PDAC murine model. Kelsey E. Huntington[1], Anna Louie[1], Young Lee[1], Jared Mompoint[1], Isacco Ferrarini[2], Aakash Jhaveri[3], Varun V. Prabhu[4], Allen Melemed[4], Seulki Lee[5], Wafik S. El-Deiry[1]. [1]Brown University, Providence, RI, [2]University of Verona, Verona, Italy, [3]Sidney Kimmel Medical College, Philadelphia, PA, [4]Chimerix, Durham, NC, [5]D&D Pharmatech, Gaithersburg, MD.

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive and lethal cancer with a 4.2% 5-year survival rate. There is an urgent need for innovative treatments for patients suffering from PDAC. Patients with PDAC often have immunosuppressed tumors that are apoptosis-resistant and have limited immune cell infiltration and/or activation. Tumor necrosis factor-Related Apoptosis-Inducing Ligand (TRAIL) selectively induces cancer cell apoptosis during innate immunity, while ONC212 is a potent fluorinated second-generation imipridone currently in IND-enabling studies that induces TRAIL signaling and the integrated stress response (ISR). TLY012 is a novel PEG'ylated trimeric TRAIL receptor agonist being clinically developed to overcome the short half-life of TRAIL. We hypothesized that combining a TRAIL pathway inducer and a TRAIL receptor agonist may be efficacious in PDAC and may activate immune cell killing based on prior work with imipridone ONC201. Immune cell co-culture experiments were conducted using PANC1 PDAC cells and TALL-104 CD8+ cytotoxic T-cells at a 1:1 effector-to-target cell ratio with or without ONC212. We observed a significant increase in T-cell killing of PDAC cells in the co-culture assay following treatment with ONC212 at several doses as compared to controls. We noted an increase in tumor-immune cell interactions as measured by the number of T-cells that were directly touching tumor cells at each timepoint. The BxPC-3 immunodeficient murine PDAC model revealed an increase in natural killer (NK) cell tumor infiltration by immunohistochemistry (IHC) staining for NK1.1 after 30 days of 50 mg/kg ONC212 treatment three times a week. The combination of ONC212 and TLY012 was evaluated using subcutaneously-implanted KPC-Luc PDAC cells in a syngeneic immunocompetent C57BL/6 mouse model. Mice treated with 25 mg/kg ONC212 alone twice a week, 10 mg/kg TLY012 alone twice a week, or combination therapy with ONC212 and TLY012 showed statistically significant decreases in tumor growth compared to controls after 5 weeks of treatment (n=6 mice per treatment condition) by tumor volume and *in vivo* bioluminescent imaging. We are analyzing murine plasma cytokine profiles, changes in tumor-infiltrating NK- and T-cells by flow cytometric and IHC analysis of murine spleen and KPC-Luc tumor samples. We report a novel immune stimulation of the TRAIL pathway and T-cell activation by ONC212 plus TLY012 leading to cytotoxic anti-PDAC responses *in vivo*.

PO-065 SIWA318H, an advanced glycation end product (AGE) targeting antibody, is efficacious in a humanized mouse xenograft model for pancreatic cancer. Ashley Jensen[1], Gabriela R. Rossi[2], Ruben Muñoz[1], Kimberly Brothers[1], Lewis Gruber[2], Misty Gruber[2], Haiyong Han[1]. [1]Translational Genomics Research Institute, Phoenix, AZ, [2]SIWA Therapeutics, Inc., Chicago, IL.

SIWA318H is a novel monoclonal antibody that selectively targets a biomarker (an advanced glycation end product) found on the surface of cells exhibiting a combination of: (a) an abnormally high level of aerobic glycolysis, a process known to be associated with initiation and progression of cancer as well as metastasis; and (b) oxidative stress. Cells with this biomarker are often dysfunctional and associated with aging, cancer, and infectious diseases. In this study, we evaluated the antitumor activity of SIWA318H in a humanized xenograft model for pancreatic cancer. SIWA318H was found to react to pancreatic cancer cells (PSN1) and cancer associated fibroblasts (CAF08) using immunofluorescent staining. SIWA318H also showed strong reactivity with tumor xenografts derived from patients with pancreatic cancer as demonstrated by immunohistochemistry. Humanized $CD34^+$ NSG mice were injected subcutaneously with PSN1 pancreatic cancer tumor cells. Ten days later, mice were randomized and enrolled in one of the following groups (n=16/group): 1) IgG1 isotype control (20mg/kg, BIWx1 followed by 10 mg/kg, BIWx2); 2) high dose SIWA318H (20mg/kg, BIWx1 followed by 10 mg/kg, BIWx2); and 3) low dose SIWA318H (10 mg/kg, BIWx1 followed by 5mg/kg, BIWx2). Tumors in mice treated with SIWA318H grew significantly slower than those in mice treated with isotype control antibody ($p<0.001$). After 3 weeks of treatment with the high dose or the low dose of SIWA318H, the tumor growth was suppressed by 68.8% and 61.5%, respectively, when compared to the isotype antibody control (ANOVA $p<0.002$). Moreover, a significant increase in complete remission (CR) rate was observed in mice receiving high (60%, $p<0.05$) or low dose (77.8%, $p<0.02$) of SIWA318H treatment compared with the control mice (6.7%). No significant antibody treatment related weight loss in mice was observed. These results provide compelling evidence that SIWA318H is a promising novel therapeutic for pancreatic cancer. Additional studies to better understand the mechanism of action of this novel immunotherapy agent are underway.

PO-066 High uptake, retention, and in vivo activity of L-Annamycin in pancreatic cancer models. Ya'an Kang, Rafal Zielinski, Roberto Cardenas Zuniga, Radjendirane Venugopal, Maria Poimenidou, Magdalena Remiszewski, Shaohua Peng, Edd Felix, Krzysztof Grela, Stanislaw Skora, Van N. Nguyen, Izabela Fokt, Waldemar Priebe. UT MD Anderson Cancer Center, Houston, TX.

Introduction: Annamycin (ANN) is a non-cardiotoxic potent topoisomerase II poison that is structurally related to doxorubicin (Dox) but it displays significantly different biological properties. ANN is formulated in multilamellar liposomes to enable effective in vivo administration as L-ANN. Pancreatic ductal adenocarcinoma (PDAC) represents a major challenge and is the deadliest cancer in decades. Pancreas drug uptake and retention remains a significant factor contributing to the chemoresistance of PDAC. Additionally, drug resistance on a cellular level further limits the therapeutic options for pancreatic cancer patients. Based on unique biological properties, we select L-ANN for detailed characterization of anti-tumor potency in preclinical PDAC models. **Objective:** The objective of this study was to validate the

L-ANN exceptional uptake in pancreatic cancer cells and tumors and explore its high pancreas uptake to target PDACs by assessing the efficacy of L-ANN in the preclinical mouse models. **Methods:** The uptake, cytotoxicity, and L-ANN-induced apoptosis were studied in human MDA-PATC50 and MDA-PATC53 cell lines. L-ANN pharmacokinetics and tissue-organ distribution were analyzed in CD-1 mice after bolus administration of L-ANN, ANN or DOX. Subcutaneous and orthotopic MDA-PATC50 and MDA-PATC53 mouse models were used to assess activity of L-ANN *in vivo*. **Results:** Fluorescent microscopy and FACS analysis showed significantly higher uptake of ANN in MDA-PATC50 and MDA-PATC53 when compared to DOX-treated cells. Interestingly, ANN displayed distinct subcellular distribution with predominantly cytosolic localization. Average IC_{50} of ANN or DOX treated MDA-PATC50 and MDA-PATC53 cells were 25.1 *nM* and 41.9 *nM* for ANN and 32.8 nM and 130.4 nM for DOX respectively (72h exposure). Biodistribution study showed rapid L-Ann absorption in the pancreas. The C_{max} of L-Ann was 15-fold higher than Dox after administration of L-ANN or Dox (both at 4 mg/kg) in pancreatic tissue. The subcutaneous models demonstrated dose-dependent reduction in tumor volumes after 3 doses of L-Ann in both PDAC models. Additionally, efficacy of L-ANN was assessed in two orthotopic PDAC models. MRI confirmed tumor volume reduction in L-Ann 3 mg/kg and 4 mg/kg doses in MDA-PATC50 model ($p<0.05$ for both 3 and 4 mg), and in MDA-PATC53 model ($p<0.001$ and $p<0.0001$, respectively). The median survivals (MS) were 135 and 103 days for L-Ann at 3 mg/kg and 4 mg/kg respectively (MS for vehicle was 49 days, $p<0.0001$). MDA-PATC50 orthotopic model demonstrated the similar results (ongoing study). **Conclusion:** Annamycin exhibits high and rapid uptake in the pancreatic cancer cells and in pancreas itself when compared to Dox. Subsequently, ANN appeared to be highly cytotoxic against tested pancreatic cancer cell lines and as L-ANN potently inhibits tumor growth and increase survival in experimental models of pancreatic cancer. These experiments demonstrate remarkable activity of L-ANN in PDAC models and support future clinical studies.

PO-067 A multi-omics study in patient-derived organoids reveals MNX1-HNF1B axis to be indispensable for intraductal mucinous papillary neoplasm lineages. Hiroyuki Kato[1], Keisuke Tateishi[1], Keisuke Yamamoto[1], Dousuke Iwadate[1], Hiroaki Fujiwara[2], Takuma Nakatsuka[1], Koji Miyabayashi[1], Yotaro Kudo[1], Ijichi Hideaki[1], Kazuhiko Koike[3], Mitsuhiro Fujishiro[1]. [1]Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan, [2]Division of Gastroenterology, The Institute for Adult Diseases, Asahi Life Foundation, Tokyo, Japan, [3]Department of Gastroenterology, Kanto Central Hospital, Tokyo, Japan.

Chromatin architecture governs cell lineages by regulating the specific gene expression; however, its role in the diversity of cancer development remains unknown. Among pancreatic cancers, pancreatic ductal adenocarcinoma (PDAC) and invasive carcinoma with an associated intraductal papillary mucinous neoplasms (invasive IPMNs) arise from two distinct precursors, and their fundamental differences remain obscure. Here, we hypothesized that chromatin profiles may clarify their intrinsic molecular features. We originally established 28 human organoids from distinct subtypes of pancreatic tumors, including IPMN, invasive IPMN, and PDAC as well as from normal ductal cells and performed exome-seq, RNA-seq, ATAC-seq, ChIP-seq, Hi-C, and phenotypic analyses with shRNA or CRISPR interference. Established organoids successfully reproduced the histology of primary tumors. IPMN and invasive IPMN organoids specifically harbored *GNAS*, *RNF43*, and *KLF4* mutations and showed the distinct lineage

related expression profiles compared to PDAC. In addition, chromatin accessibility profiles well stratified the respective tumor groups. Notably, this analysis supported the histological features of tumor subtypes; gastric IPMN gained the stomach-specific accessible regions and the accessible pattern of invasive IPMN linked to diverse gastrointestinal tissues. In contrast, PDAC was characterized by the significant loss of chromatin accessibility seen in normal pancreatic ductal cells. Footprint analysis of transcription factors (TFs) identified specific TFs that are enriched in each tumor subtype. Of note we found the footprint of HNF1B to be active in IPMN lineages but not in PDAC. To address its biological significance, we analyzed the effects of HNF1B by knockdown (KD) experiments and revealed that HNF1B is biologically indispensable for IPMN lineages. We further identified MNX1 as an upstream regulator of HNF1B expression, another TF expressed in multipotent pancreatic progenitor cells. ChIP experiment revealed the enriched binding of MNX1 on the promoter elements of *HNF1B* in invasive IPMN. Importantly, KD or CRISPR interference of MNX1 impaired the survival of the organoids from invasive IPMN. Moreover, the correlated and high expression patterns of MNX1 and HNF1B in IPMN lineages were validated in a set of human tissues. To identify the common downstream genes of MNX1 and HNF1B, we analyzed RNA-seq and ATAC-seq after KD of the two genes. We found that MNX1-HNF1B axis governed a set of genes including *MYC*, *SOX9*, and *OLFM4*, which are known to regulate stem cell properties in gastrointestinal epithelium. Lastly, to get a broader view of chromatin architecture in these tumors, we performed Hi-C. We found that HNF1B target genes to be three-dimensionally condensed in the genome of invasive IPMN but not in that of PDAC, supporting the functional importance of those genes in invasive IPMN. Collectively, our organoid analyses unraveled the different lineage related chromatin structures correlating to the specific biological behaviors in pancreatic tumor subtypes.

PO-068 Cholesterol auxotrophy promotes the expansion of centroacinar cells giving rise to the basal subtype of pancreatic adenocarcinoma. Michael Kotliar[1], Aizhan Surumbayeva[2], Linara Gabitova[2], Suraj Peri[3], Diana Restifo[2], Kathy Q. Cai[4], Artem Barski[5], Igor Astsaturov[2]. [1]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, [2]The Marvin & Concetta Greenberg Pancreatic Cancer Institute, Fox Chase Cancer Center, Philadelphia, PA, [3]Biostatistics and Bioinformatics Facility, Fox Chase Cancer Center, Philadelphia, PA, [4]Histopathology Facility, Fox Chase Cancer Center, Philadelphia, PA, [5]Cincinnati Children's Hospital Medical Center and Department of Pediatrics, University of Cincinnati, Cincinnati, OH.

Gene expression analyses established at least two molecular subtypes of pancreatic adenocarcinoma (PDAC), the classical (or glandular), and the basal (or mesenchymal), each of which is associated with distinct prognoses and sensitivity to chemotherapy. It remains unclear, however, whether the basal carcinoma cells arise from a separate cell-of-origin, or are emerging from the pre-existing well-differentiated "classical" PDAC cells. To distinguish these alternatives, we conducted single-cell transcriptome analyses and virtual lineage tracing comparing cellular populations at pre-malignant stages in basal versus classical PDAC mouse models. We previously reported that chemical or genetic inhibition of the cholesterol biosynthetic pathway in *Kras^{G12D}; Trp53* (*KPPC*) mice predisposes to basal rather than glandular PDAC development because of the pancreas-specific increased sterol response element-binding protein 1 (SREBP1) activity and TGFβ signaling that induces cancer cell stemness and the EMT (PMID: 32976774). Pancreas-selective knockout of a conditional allele of cholesterol pathway gene, *Nsdhl* (NAD(P)-dependent steroid dehydrogenase-like), renders pancreatic epithelial cells

cholesterol auxotrophs and drives basal PDAC in the majority of animals (*KPPCN* mice). At 5-6 weeks of age, grossly and microscopically tumor-free pancreatic tissues were selected for single-cell isolation and single-cell RNA sequencing (scRNA seq) using the 10X platform. After standard filtering and sample normalization procedures, downstream analyses included identification of relevant cell clusters using Seurat, lineage tracing algorithms, and in silico modeling of autocrine and paracrine signaling interactions between subsets of PDAC and non-malignant cells. Our key findings are as follows: 1) premalignant *KPPCN* pancreata exhibit a massive expansion of cancer-associated fibroblasts (CAFs) of predominantly inflammatory differentiation (iCAFs); 2) despite relatively fewer ADM and PanIN pre-malignant lesions in *KPPCN* compared to *KPPC*, scRNA seq identifies the significant expansion of epithelial cells with features of centroacinar and stem-like cells (increased expression of *Aldh1a2, Nes, Sox9, Ly6a, Cxcl12, and Met*); these centroacinar-like cells, while retaining epithelial identity (*Epcam, Cdh1*), also exhibit features of pluripotency by co-expression of *Ins2* and other stem cell markers; 3) alignment with basal PDAC (*KPPCN*) and classical (*KPPC*) carcinoma cell populations strongly suggests the continuity of clonal evolution of the centroacinar-like cells towards the basal PDAC. While our genetic model does not recapitulate the multiple alternative pathways leading to basal PDAC development, cholesterol auxotrophy via SREBP1 may be a factor governing the expansion of undifferentiated precursors, which via interactions with cancer-promoting iCAFs, drive basal PDAC development.

PO-069 Modeling the tumor microenvironment using tissue engineering technologies.

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Tissue engineering technologies provide controllable and reproducible approaches to reconstruct the extracellular and cellular elements of pancreatic cancer. In a reductionist approach they allow the modelling of the complex tumor microenvironment (TME) and the study of disease biology and anti-cancer treatments. Our objective is that an engineered TME model, that mimics the tissue and matrix composition, architecture and cell types, will behave like a real tumor and is suitable for preclinical drug testing. Using biomimetic materials, we recreated the desmoplastic tissue characteristics of the pancreatic TME. Our natural and synthetic biomaterials were tailored to achieve the mechanical properties that resembled the stiffness and viscoelasticity of patient-derived tissues, providing a supportive cell-matrix interface for 3D cell culture conditions. Pancreatic cancer cells grown embedded in the matrix scaffolds formed tumor spheroids. Cell-based assays and microscopic analysis indicated a high cell viability and proliferation of the tumor spheroids as well as the expression of cancer-associated markers. Incorporation of cancer-associated fibroblasts and myeloid cells led to a multicellular 3D systems and matrix stiffening due to the secretion of extracellular matrix proteins. Transcriptomic analysis of the 3D cell cultures identified differentially regulated pathways related to cell proliferation, mechano-transduction and the secretion of pro-inflammatory cytokines, indicative of a malignant behavior. Treatment with mechano-modulating inhibitors and anti-cancer compounds increased the efficacy of chemotherapeutics, thereby reducing matrix stiffness and the release of cytokines. Our engineered TME model provides an easily translatable technology to ease the burden of pancreatic cancer, allowing us to characterize combination treatments that slow down or reduce tumor growth.

PO-070 Longitudinal precision oncology platform to identify chemotherapy-induced vulnerabilities in pancreatic cancer. Katja Peschke[1], Hannah Jakubowski[1], Arlett Schäfer[1], Carlo Maurer[1], Sebastian Lange[1], Felix Orben[1], Raquel Bernad[1], Felix Harder[1], Matthias Eiber[1], Rupert Öllinger[1], Melissa Schlitter[1], Wilko Weichert[1], Veit Phillip[1], Christoph Schlag[1], Roland Schmid[1], Rickmer Braren[1], Bo Kong[2], Ekin Demir[1], Helmut Friess[1], Roland Rad[1], Dieter Saur[1], Günter Schneider[1], Maximilian Reichert[1]. [1]Technical University of Munich, Klinikum rechts der Isar, Munich, Germany, [2]University of Ulm, Ulm, Germany.

Pancreatic ductal adenocarcinoma (PDAC) remains a devastating disease with poor survival rates as almost all patients develop resistance towards chemotherapy and molecular-informed targeted therapies are reserved to a few. Here, we aim to establish a longitudinal precision oncology platform with a multi-dimensional characterization of PDAC biopsies including genomic, transcriptomic as well as functional analyses to identify and exploit treatment-induced vulnerabilities. In order to investigate adaptive processes of tumors under treatment we aimed to generate PDAC patient-derived organoids (PDOs) and 2D cell lines before and after chemotherapy. Therefore, we enrolled a patient with borderline resectable PDAC who received neoadjuvant FOLFIRINOX. Endoscopic fine needle (pre-FFX) and surgical biopsies (post-FFX) were used to generate PDOs and 2D cells. Whole exome sequencing (WES) and RNA sequencing data of the pre-FFX and post-FFX organoids were compared in order to evaluate the genetic landscape and PDAC subtypes. 2D cells were subjected to an unbiased automated drug screening of 415 compounds to investigate FFX-induced vulnerabilities. Top targets were validated manually in the 2D cells and organoids. Although transcriptional subtyping classified both PDOs as classical PDAC, gene set enrichment analysis (GSEA) revealed a reduced pathway activation linked to the basal-like phenotype such as KRAS signaling in the post-FFX organoids. WES did not show major differences in the genetic landscape of the tumor pre- and post-FFX induction suggesting a plasticity process rather than a clonal selection during chemotherapy. Importantly, post-FFX cells exhibited an increased sensitivity in the unbiased drug screening towards MEK and EGFR inhibition compared to pre-FFX cells. 2D cells and organoids were treated with different MEK inhibitors (MEKi) for validation and post-FFX cells showed a highly increased response compared to the treatment-naïve cells, as well. Interestingly, when placed into the context of a panel of 15 primary PDAC cell lines the pre-FFX cells cluster with highly MEKi resistant PDAC cells whereas post-FFX cells belong to the most sensitive cell lines. In sum, integrating functional layers into personalized medicine allowed us to identify chemotherapy-induced vulnerabilities as potent targeted therapy options in PDAC. Thus, this longitudinal precision oncology platform harbors a unique opportunity to understand adaptive processes in tumor evolution and/or treatment-imposed pressure in PDAC patients.

PO-072 Inhibiting vasoactive intestinal peptide receptor signaling elicits T cell dependent anti-tumor response of pancreatic ductal adenocarcinoma to immune checkpoint therapy. Sruthi Ravindranathan[1], Passang Tenzin[1], Jian Ming Li[1], Rohan Dhamsania[1], Michael Ware[1], Mohammad Zaidi[1], Shuhua Wang[1], Jingru Zhu[1], Maria Cardenas[1], Yuan Liu[1], Gaurav Joshi[1], Sanjeev Gumber[1], Brian Robinson[1], Anish Sen-Majumdar[2], Shanmuganathan Chandrakasan[1], Haydn Kissick[1], Alan Frey[2], Susan Thomas[3], Bassel El-Rayes[1], Gregory Lesinski[1], Edmund K. Waller[1]. [1]Emory University, Atlanta, GA, [2]Cambium Oncology, Atlanta, GA, [3]Georgia Institute of Technology, Atlanta, GA.

Pancreatic ductal adenocarcinoma (PDAC) is unresponsive to immune checkpoint therapy largely due to a paucity of T cells within the tumor microenvironment (TME) and abundant immunosuppressive signaling pathways. In this study we show that human PDAC tumors over-express vasoactive intestinal peptide (VIP), an immunosuppressive neuropeptide that suppresses T cell effector properties and promotes the generation of regulatory T cells (Tregs). Therefore, we treated tumor-bearing mice with VIP receptor peptide antagonists and measured T cell homing, activation, and anti-tumor responses in preclinical murine models of PDAC. Pharmacological inhibition of VIP receptor (VIP-R) signaling using daily subcutaneous injections of peptide antagonists had no discernable toxicity in healthy and tumor-bearing mice. Treatment with VIP-R antagonists in combination with anti-PD-1 checkpoint blockade significantly decreased tumor burden, and improved survival in subcutaneous and orthotopic murine PDAC models. Combination therapy significantly enhanced T cell activation and proliferation and decreased frequencies of Tregs within the TME. Anti-tumor responses were T cell dependent, as the combination therapy failed to improve survival in CD4 or CD8 deficient mice using knock-out strains and antibody depletion. Furthermore, combination therapy significantly increased frequencies of tumor specific T cells (measured with a tetramer reagent) and provided protective immunity against tumor rechallenge. Combination therapy led to significant increases in the infiltration of adoptively transferred GFP+ T cells into PDAC tumors and decreased CXCR4 expression levels on T cells. Encouragingly, peptide-based VIP-R antagonists enhanced the *in vitro* activation of human T cells isolated from peripheral blood of PDAC patients. Human T cells cultured with VIP-R antagonists had increased proliferation, activation, and decreased proportions of T regs and exhausted T cells co-expressing PD-1, Tim-3 and Lag-3. Taken together, our findings show that VIP is a targetable mechanism of immune escape in PDAC. Inhibiting VIP receptor signaling improves T cell effector properties and synergistically improves anti-tumor responses to checkpoint inhibitors in mouse PDAC models. Additionally, as the VIP sequence is identical between human and mice, and since VIP-R antagonists have similar effects on human and murine T cells in culture, clinical translation is highly feasible.

PO-073 Inactivation of Notch4 attenuated pancreatic tumorigenesis in mice. Kiyoshi Saeki[1], Wanglong Qiu[1], Richard Friedman[1], Carrie Shawber[1], Jan Kitajewski[2], Jianhua Hu[1], Gloria H. Su[1]. [1]Columbia University Irving Medical Center, New York, NY, [2]University of Illinois Chicago, Chicago, IL.

Expression of the Notch family of receptors are often upregulated in pancreatic ductal adenocarcinoma (PDAC), however, the functional impacts of the Notch signaling network on pancreatic tumorigenesis remain unresolved. In this study, we focused on Notch4, which had not been investigated in PDAC. Leveraging the conventional Notch4 deficient mouse line and previously established genetically engineered mouse models (GEMM) for PDAC, we generated KC (*LSL-Kras^{G12D};p48-Cre*), N4$^{-/-}$KC (*Notch4$^{-/-}$;LSL-Kras^{G12D};p48-Cre*), PKC (*p16$^{flox/flox}$;LSL-Kras^{G12D};p48-Cre*), and N4$^{-/-}$PKC GEMMs (*Notch4$^{-/-}$; p16$^{flox/ lox}$;LSL-Kras^{G12D};p48-Cre*). We performed caerulein treatment in both KC and N4$^{-/-}$KC mice, and compared the development of acinar to ductal metaplasia (ADM) and pancreatic intraepithelial neoplasia (PanIN) between them. The ADM/PanIN lesions were significantly smaller in the N4$^{-/-}$KC than in the KC GEMM (p=0.01), suggesting that Notch4 deficiency attenuated early pancreatic tumorigenesis. This *in vivo* result was confirmed by *in vitro* ADM induction of the explant cultures of mouse pancreatic

acinar cells. The number of ADM structures in the $N4^{-/-}$KC acinar cultures was significantly lower than the KC acinar cultures ($p<0.001$). To evaluate the role of Notch4 in the later stage of pancreatic tumorigenesis, we compared the histological progression and overall survival between the PKC and $N4^{-/-}$PKC mice. We found that $N4^{-/-}$PKC mice had better prognosis ($p=0.012$) and less tumor burden (PanIN: $p=0.018$ (2 months), PDAC: $p=0.039$ (5 months)) compared to the PKC GEMM. RNA-Seq analysis of pancreatic tumor cell lines derived from the PKC and $N4^{-/-}$ PKC GEMMs revealed 408 genes were differentially expressed (FDR<0.05) and the genes related to the NGF processing as novel downstream effectors of the Notch4 signaling pathway($p<0.001$). Our study is a novel biological investigation that demonstrated that Notch4 signaling possesses tumor promoting function in pancreatic tumorigenesis. Our study revealed a novel association between the NGF processing pathway and Notch4 signaling in PDAC.

PO-074 Identification of C-MET receptor as a therapeutic target in patient-specific tumoroid models of metastatic pancreatic adenocarcinoma allows identification of a new mode of action for its inhibitors. Liam Deems, Maria Ivanova, Cheryl Murphy, Amit Shahar, David Deems, Dmitry Shvartsman. Cellaria Inc., Wakefield, MA.

Creating patient-specific models with a high level of characterization and functionality in tumoroid modeling enables a better understanding of the disease drivers in metastatic cancers. It presents the opportunity for personalized treatment with novel therapeutic reagents in underrepresented cancers such as pancreatic ductal adenocarcinoma. Using a defined and treatment-naive patient-specific model cohort of nine patients diagnosed with pancreatic adenocarcinoma, we aimed to represent the diversity of progression grade and assay profiles. These models were tested with a panel of common, broad-range chemotherapeutic agents and characterized for genetic, proteomic, and tumoroid drug response profiles. A detailed analysis of gene expression values from the original tissue specimen, compared with cancer cultures in hypoxic, normoxic planar, and spheroid (3D) culture conditions, enabled the identification of C-MET as a critical gene that shows upregulation closely resembling the expression profile observed in the original patient biopsy and was modulated by tissue culture format. Once this potential target was identified, the pancreatic models were treated with five C-MET-targeting chemotherapeutic agents with different modes of action. Proto-oncogene receptor C-MET (HGF receptor) is a potential therapeutic target in many metastatic cancers due to its integral role in angiogenesis, proliferation, and cancer cell survival[1]. We propose that these reproducible and easily scaled tumoroid models can be used to find new therapeutic targets in-vitro and provide the example of C-MET in our pancreatic cancer patient cohort. Moreover, only 3D hypoxic models closely resembled original patient tissues in their genomic expression profiles. Using these model formats, we distinguished differences between various C-MET targeting compounds and their ability to induce cell death, which correlated with their mode of action on C-MET neutralization. Identifying these targets in 3D produces new and potentially more effective drug targets where they may not have been observed solely in planar conditions with legacy cell lines. Our models of metastatic pancreatic cancer tumoroids can be readily utilized to benefit researchers and clinicians seeking new targets for patient treatment.

PO-075 The elucidation of the role of Prrx1 for acinar to ductal metaplasia in response to acute injury of pancreas in the novel mouse models. Kensuke Suzuki[1], Alina Li[1], Jason R. Pitarresi[1], Anna M. Chiarella[1], Gizem Efe[1], Kensuke Sugiura[1], Rohit Chandwani[2], Anil K.

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Introduction: Pancreatic acinar cells can de-differentiate after acute injury (acute pancreatitis) to a progenitor-like cell type with ductal characteristics in a process termed acinar-to-ductal metaplasia (ADM). In the absence of oncogenic mutation, the ADM lesions resolve and reform the acinar compartment (adaptive ADM). However, in the presence of oncogenic *Kras* mutations (oncogenic ADM), ADM lesions can evolve to pancreatic intraepithelial neoplasia (PanIN) and pancreatic ductal adenocarcinoma (PDAC). Our comprehensive and unbiased approach previously identified the Paired-Related homebox1 (Prrx1) as the most up-regulated transcription factor during pancreatic development, regeneration and evolution of PanIN. We showed that *Prrx1* expression is upregulated in both ADM and PanIN lesion. Here, we explore the role of Prrx1 in ADM and PanIN formation using novel mouse models, *ex vivo* acinar culture systems, and human pancreatitis tissue microarrays (TMA). **Methods:** We generated novel $Ptf1a^{cre\text{-}ERT}$;$Prrx1^{fl/fl}$;$Rosa26^{YFP/YFP}$ (Prrx1 KO) mice, in which *Prrx1* is deleted and YFP is expressed exclusively in adult pancreatic acinar cells upon tamoxifen induction of Cre recombinase activity. Intraperitoneal caerulein injection was administered to induce acute pancreatitis. Human pancreatitis TMAs were utilized for the comparison of Prrx1 expression between pancreatitis and normal human pancreas. We also generated Pdx1*Cre*;*LSL-KrasG12D/+*; $Prrx1^{fl/fl}$;$Rosa26^{YFP/YFP}$ (KCYPrrx1KO) mice to evaluate the function of Prrx1 in oncogenic *Kras* mutation induced pancreatic PanIN. Quantification of ADM regions was performed through histological examination by a pathologist (blinded to the genetic basis of the tissues) and automated cell-counting of immunofluorescence staining (IF). Dissociated acinar cell culture in collagen was utilized for the evaluation of ADM under *ex vivo* conditions. We also established a novel method for the induction of Prrx1 overexpression in dissociated acinar cells via nucleofection. **Results:** IF staining revealed that Prrx1 expression is efficiently deleted in ADM cells of Prrx1 KO mice 3 days post-caerulein treatment, a timepoint at which we see peak ADM region formation. Additionally, areas of ADM lesions and amylase loss were significantly reduced in Prrx1 KO mice compared with Prrx1 WT mice at day 3. We also found that Prrx1 overexpression promotes ADM formation in *ex vivo* acinar cell cultures. In human TMAs, pancreatitis tissues had higher expression of Prrx1 than in normal pancreas. In the presence of oncogenic *Kras* mutation, loss of Prrx1 significantly attenuates ADM formation in *ex vivo* culture. Moreover, 5-month-old KCYPrrx1KO mice have very limited ADM/PanIN region compared to 5-month-old KCY mice, which have a pancreas that has predominantly replaced the ADM/PanIN. **Conclusions:** Prrx1 promotes ADM formation in both adaptive ADM and oncogenic ADM. Our preliminary data suggest that Prrx1 facilitates PDAC progression through PanIN formation.

PO-076 Murine adapted FOLFIRINOX for standard-of-care in KPC mice. Martin C. Whittle, Aditi Palkar, Rachael Fasnacht, James Yan, Borith Kheng, Kianna Sinfuego, Shelley Thorsen, Sunil R. Hingorani. Fred Hutchinson Cancer Research Center, Seattle, WA.

Until relatively recently, the standard-of-care (SOC) treatment for pancreas cancer was not appreciably superior to best supportive care in prolonging life, and potentially new treatment strategies had a relatively modest bar to surmount. New combination regimens have emerged and

novel therapies should therefore be compared against the current SOC. However, in preclinical development of novel agents, efficacy is frequently compared against vehicle control and less commonly against cytotoxic chemotherapy. Furthermore, virtually all studies of cytotoxic chemotherapy for PDA in mice have used gemcitabine (gem) monotherapy as standard-of-care, although this is no longer the standard for this disease. One of the current standard regimens, gem+nab-paclitaxel, cannot be given in mice without inducing immune responses because of the incorporation of human albumin in developing the taxol nanoparticles. The other new standard, FOLFIRINOX, is a complex 4-agent regimen that includes a 46-hour continuous infusion component that has been prohibitively challenging to deliver to animals. We have now built an appropriate dosing regimen incorporating these 4 agents that required a deliberate, step-by-step, program of empiric testing of the drugs, first, one at a time, then in various doublets and triplets, and, finally, as a whole regimen. The studies included close clinical evaluations and serial blood tests to follow complete blood counts (CBC). We have incorporated this regimen as the new SOC in our Murine Clinical Trials Program (MCTP). We propose that this mur-FOLIFIRINOX regimen should represent the new standard for murine studies.

PO-077 Establishment of a novel living biobank of patient-derived pancreatic cancer organoids with genomic and drug response characterization. Irene Y. Xie[1], Laura Tamblyn[2], Karen Ng[2], Eugenia Flores-Figueroa[2], Julie M. Wilson[3], Gun Ho Jang[3], Amy X. Zhang[3], Stephanie Ramotar[2], Anna Dodd[2], Nikolina Radulovich[2], Jennifer J. Knox[2], Grainne M. O'Kane[2], Steven Gallinger[2], Faiyaz Notta[2]. [1]University of Toronto, Toronto, ON, Canada, [2]University Health Network, Toronto, ON, Canada, [3]Ontario Institute for Cancer Research, Toronto, ON, Canada.

Advanced pancreatic cancer has a dismal prognosis and current treatment options (FOLFIRINOX, Gemcitabine/nab-paclitaxel [GnP]) are associated with toxicity. Although some patients achieve partial responses, most progress rapidly and become chemorefractory. While RNA subtypes, genomic alterations, and protein biomarkers have prognostic value, predictive biomarkers to guide therapy are needed. Patient-derived organoids (PDOs) are an increasingly popular model for predicting patient responses to standard-of-care therapy and investigating personalized therapy options. We present a novel biobank of 42 PDOs and drug profiling data with 5 standard of care agents and 3 kinase inhibitors. Tissue was processed from n=103 biopsies from 97 patients with a confirmed pathologic diagnosis of advanced (Stage III-IV) pancreatic ductal adenocarcinoma who presented to a single Canadian tertiary care centre between 2017-2020. Matched WGS was available in all cases. Our PDO generation success was 42/103 (41%). We observed a trend towards decreased establishment in tumors that were *KRAS* WT, *TP53* WT, or had higher HRDetect scores. Conversely, polyploidy, *SMAD4* WT, and major imbalances in mutant *KRAS* were associated with successful PDO establishment. These associations were not statistically significant after multiple comparisons correction, but suggest selection for success with more aggressive tumors. Drug profiling was performed on all 42 PDOs with the individual agents of FOLFIRINOX (5-FU, irinotecan, oxaliplatin), GnP (gemcitabine, paclitaxel), and three targeted agents (afatinib, trametinib, and talazoparib). Combination testing was also performed for gemcitabine + paclitaxel. Drug responses were measured through both viability and growth rate (GRMetrics). We found that GRMetrics minimized effects from different PDO growth rates. Matched clinical data were available for 23 patients who received FOLFIRINOX, 11 patients who received GnP, and one patient who received gemcitabine monotherapy. Similar to previous

studies, we found that *in vitro* PDO responses to 5-FU, irinotecan, and GnP were correlated with patient responses based on RECIST criteria. Interestingly, and similar to previous reports in colorectal cancer PDOs, we found that oxaliplatin responses were not predictive of RECIST response. As expected, PDOs were resistant to afatinib (EGFRi), which reflects negative clinical trials, and may also be masked by use of EGF in growth media. A range of responses to trametinib (MEKi) were seen but were not correlated with KRAS allelic dosage. A similar range of response was seen to talazoparib (PARPi), but did not correlate with oxaliplatin response or HRDetect scores. In summary, we have established a novel biobank of PDOs from advanced pancreatic cancer patients. Notably, PDOs were less likely to establish from tumors that were *KRAS* WT or HR-deficient, even though these patients are likely to benefit from targeted approaches. Further investigation is required to develop PDO use in clinical drug prediction and drug discovery.

PO-079 Proteomic profiling reveals subtype specific kinase expression in pancreatic cancer. Yi Xu, Michael East, Ashley Morrison, Gabriela Herrera, Laura Peng, Gary Johnson, Jen Jen Yeh. UNC Chapel Hill, Chapel Hill, NC.

Pancreatic adenocarcinoma (PDAC) remains an extremely lethal disease with few effective therapeutic options. Targeted therapy development has remained largely unsuccessful due to complex tumor heterogeneity and better molecular indicators of response are urgently needed. We have previously identified two distinct molecular tumor subtypes in PDAC, basal-like and classical, which are prognostic and dictate response to chemotherapy. We have also developed a patient derived xenograft (PDX) mouse model that maintains the integrity of patient tumor biology and recapitulates primary tumor subtypes *in vivo*. Here, we aim to utilize multiplexed kinase inhibitor beads and quantitative mass spectrometry (MIB-MS) to determine baseline kinase expression and adaptive kinome responses to therapy in PDAC PDX tumors. A total of 381 kinases were identified across all samples and tumor specific kinases were isolated by alignment to human peptides. Distinct kinase expression profiles were observed for basal-like and classical tumors. Basal-like tumors showed high expression of kinases involved in receptor tyrosine kinase activity, MAP kinase activity, and clathrin-dependent endocytosis. Classical subtype tumors showed increased expression of kinases involved in metabolism and cytoskeletal regulatory kinases. Differential protein expression was further validated by differential gene expression using RNAseq. MIB profiling of kinome response to EGFR inhibition by erlotinib indicated kinome reprogramming with upregulation of microtubule associated kinases and MAP kinase family members. Overall, these results suggest that basal-like and classical subtype tumors exhibit distinct kinome profiles that present possible subtype specific vulnerabilities to kinase inhibition. Stratifying kinase inhibitor therapies based on tumor subtype could improve response to targeted therapies in PDAC.

PO-080 Patient-derived organoids and cancer associated fibroblasts as a co-culture model to explore cell type interactions in pancreatic cancer. Jacquelyn W. Zimmerman, Genevieve Stein-O'Brien, Richard A. Burkhart, Elana J. Fertig, Elizabeth M. Jaffee. Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD.

Introduction: Pancreatic ductal adenocarcinoma (PDAC) remains challenging to treat in part due to the dense desmoplastic stroma characteristic of the tumor microenvironment (TME).

Often, less than 50% of the tumor mass is composed of malignant ductal epithelial cells. The relationship between the TME and the ductal tumor is important in tumorigenesis, metastasis, and treatment resistance. Cancer associated fibroblasts (CAFs) are an abundant cell type in the TME and have a dynamic role in both promoting and inhibiting tumor progression. Efforts to target fibroblast function therapeutically have yielded mixed results. While pre-clinical data have generated some optimism that CAF inhibition may improve clinical outcomes, this has not translated to the clinical setting. One likely reason for these inconsistent findings is the evolving understanding of CAF heterogeneity. Expanding our understanding of the interactions between the epithelial tumor and CAFs may identify new modalities for modulating the TME to augment treatment efficacy and improve patient outcomes. This requires the generation of human model systems to better interrogate intercellular interactions. **Methods:** We have developed an ex vivo model system to investigate interactions between ductal epithelial tumor cells and CAFs using matched patient-derived organoids (PDOs) and CAFs extracted following dissociation of surgical specimens. Flow cytometry was used to evaluate the viability of both cellular components as well as associated proliferation and cell identity. Moreover, we have used immunohistochemistry to further characterize this system while preserving the spatial relationship of the cell populations. Most recently, we employed the MULTI-seq multiplex single cell RNA sequencing (scRNA-seq) pipeline to explore the transcriptional dynamics of this system at intervals over 96 hours by examining 4 conditions with 1,500 cells sequenced per condition per time point. **Results:** Flow cytometry demonstrated that both PDOs and CAFs remain viable in this co-culture construct and that both cell types can be reliably identified using epithelial markers to identify organoid cells and an exclusion panel to identify the CAFs. We have further validated these findings using immunohistochemistry and demonstrated that in co-culture both PDOs and CAFs remain viable and continue to proliferate. We have also demonstrated successful extraction of both cell types from the Matrigel scaffold in which they are grown for use in downstream high dimensional genomics assays. **Conclusions:** We have previously demonstrated the utility of a PDO model to explore clinically relevant biological mechanisms in PDAC. However, the addition of CAFs to this model provides a more comprehensive representation of the in vivo tumor. This ex vivo system is viable and flexible for multiple end point assays and can be used to explore relevant cellular interactions and mechanisms. Our ongoing investigation will specifically focus on PDO-CAF interactions and transcriptional dynamics over time as we interrogate the scRNA-seq data.

Signaling

PO-081 Studying MYC's contribution to replication stress at the nuclear pore. Gabriel M. Cohn, Colin J. Daniel, Daniel F. Liefwalker, Rosalie C. Sears. Oregon Health & Science University, Portland, OR.

Genomic instability is a hallmark of cancer which promotes oncogenic mutations in pancreatic ductal adenocarcinoma (PDAC), leading to more aggressive tumors with greater potential for drug resistance. Deregulation of the master transcription factor, MYC, is found in virtually all PDAC tumors and promotes genomic alterations by increasing replication stress, augmenting DNA repair pathways, and promoting cell survival. To resolve replication stress, stalled forks are trafficked to the nuclear pores where the necessary machinery accumulates. Activated MYC is spatially reorganized to the nuclear pores as well, a mechanism that is exacerbated in

cancer. Whether MYC activity at the nuclear pores contributes to resolving replication stress in PDAC remains to be known. To investigate this mechanism, we are creating genomic and proteomic tools to determine MYC's function at the nuclear pores during replication stress induced by olaparib treatment in PDAC cells. We found that olaparib significantly increases MYC accumulation at the nuclear pore in low-passage cell lines we established from PDAC patient tumors. Furthermore, we showed that MYC interacts with the nuclear pore resident SUMO protease, SENP1, which plays a key role in resolving stalled forks. Our preliminary data suggest a role for MYC resolving replication stress at nuclear pores, and could indicate MYC activity as a therapeutic target in olaparib-resistant PDAC.

PO-082 Delineating the molecular basis of early dissemination of pancreatic cancer.

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Pancreatic ductal adenocarcinoma (PDAC) is often diagnosed at advanced stages rendering the already limited therapeutic options ineffective for many patients. Earlier diagnosis permits for "curative" surgery; however, 95% of these patients experience recurrence locally or distally and die within ten years of treatment. One hypothesis that can explain high recurrence is the early dissemination of PDAC progression. At the time of surgery, systemic subclinical dissemination of predominantly dormant cells found ubiquitously within patient livers has already occurred prior to the development of overt metastatic disease or bona fide recurrent PDAC. However, remarkably little is known about early PDAC cell dissemination despite these dismal outcomes, mainly due to the lack of human models to study disease progression. We previously demonstrated the cellular reprogramming "proof-of-principle" to model human PDAC progression (designated "10-22 cells") and its use as a discovery tool for early biomarkers by unveiling a marker for stage 1 PDAC in human patients. We further analyzed transcriptomes of genetically tagged 10-22 cells progressing from pancreatic intraepithelial neoplasia (PanIN) to PDAC in mice and validated the results using the TCGA PDAC dataset, human clinical PanIN, PDAC tissues, and a well-established murine PDAC model. We found that extracellular vesicle transport and neuronal cell differentiation pathways were derepressed in the progression of PanINs to PDAC. HMG-Box Transcription Factor 1 (HBP1) emerged as a potential master factor regulating dynamically expressed genes in neuronal cell differentiation and dissemination during PDAC progression. HBP1 expression are aberrantly regulated in human and murine PanINs and PDAC tissue samples, and their mRNA expressions are inversely correlated with PDAC patients' prognosis. Ectopic overexpression of HBP1 increased proliferation and migration of normal and cancerous pancreatic cells. Conversely, knocking out HBP1 in PDAC cells decreased proliferation and migration. Altogether, our data indicate that HBP1 may confer the cell dissemination capacity in early PDAC progression. We are on the way to further dissect the

mechanisms by which HBP1 regulates pancreatic cell migration and confers neuronal migratory phenotypes and how these signaling influence PDAC progression and prognosis.

PO-084 The role of p53 in the development of pancreatic ductal adenocarcinoma.
Kathryn J. Hanson, Brittany M. Flowers, Nicholas Hughes, Hannes Vogel, Le Cong, Laura D. Attardi. Stanford University, Stanford, CA.

Pancreatic ductal adenocarcinoma (PDAC) is a very deadly cancer, with a 5-year survival rate of only 9%. This high mortality rate can be attributed to the lack of early detection methods and to tumors being resistant to treatment, in part due to a highly immunosuppressive tumor microenvironment (TME). The most commonly mutated genes in PDAC include activating mutations in *KRAS* in >90% of cases and mutations in the tumor suppressor *TP53* gene in ~72% of cases. However, how p53, which is a transcription factor, normally suppresses PDAC progression is not well understood. Understanding the pathways through which p53 acts to suppress PDAC will provide key insight into the molecular mechanisms underlying PDAC initiation and progression. Our laboratory has recently shown in genetically engineered mouse models that Kras activation, coupled with p53 inactivation, can drive PDAC from either pancreatic acinar or ductal cells, with acinar cell- and ductal cell-derived tumor signatures resembling the classical and basal-like human PDAC subtypes, respectively. To better understand how p53 loss promotes PDAC, we are focusing on the acinar cell-derived PDAC mouse model and using single-cell transcriptomic technologies to ask in which cell states and through which pathways p53 acts to suppress PDAC development, in particular as PDAC has complex interactions between tumor cells and the TME. In this model, PDAC is driven by oncogenic Kras ($Kras^{G12D}$) along with either p53 wild type ($p53^{wt}$) or p53 deficiency ($p53^{flox}$) in adult acinar cells by a tamoxifen-inducible $Ptf1a^{CreER}$ allele. In the progression of this model, acinar cells can give rise to PDAC likely through a transdifferentiation process called acinar-to-ductal metaplasia (ADM), which leads to pancreatic intraepithelial neoplasias (PanINs) and then PDAC. The initial cell fate transition, ADM, can be activated by growth factors, inflammation, and injury. We have conducted single cell RNA-sequencing (scRNA-seq) to compare early stages of tumor development in the presence and absence of p53. We have also conducted spatial transcriptomic analysis of these samples. Using these combined approaches, we are analyzing tumor cell populations and cells of the tumor microenvironment in the $p53^{wt}$ and $p53^{flox}$ genotypes and have found. We will present our latest single cell analyses on p53 dependent cell states and functional pathways during PDAC development. Understanding how tumor cell states and cells of the tumor microenvironment change with p53 status will reveal different paths of PDAC evolution and uncover novel approaches to improve clinical intervention for human PDAC.

PO-088 Classification based on efficiency of mRNA translation reveals a metabolically-dependent subtype of pancreatic cancer.
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Sweden.

Molecular profiling of Pancreatic Ductal Adenocarcinoma (PDA), based on transcriptomic analyses, identifies two main prognostic subtypes (basal-like and classical), but does not allow personalized first-line treatment. To date, tumors have not been profiled based on protein synthesis rates, yet the step of mRNA translation is highly dysregulated in both PDA cancer cells and their microenvironment. We aim at assessing whether quantification of mRNA translation could provide a distinct perspective on PDA and identify novel tumor subtypes. Using a collection of twenty-seven pancreatic Patient-Derived Xenografts (PDX), we performed transcriptome-wide analysis of translated mRNA (translatome). Unsupervised bioinformatics analysis allowed PDA tumors classification according to mRNA translation rate. PDX-derived cancer cells as well as common PDA cell lines were used to functionally characterize newly identified subtype. Independent component analysis revealed a new tumor subtype harboring a low protein synthesis rate, but associated with a robust translation of mRNAs encoding effectors of the integrated stress response (ISR), including the transcription factor ATF4. Functional characterization of the "ISR-activated" human cancer cells revealed a high resistance to drugs, low autophagic capacities, and importantly, metabolic impairments in the serine synthesis and transsulfuration pathways. Therefore, the drug-resistant cancer cell phenotype showing auxotrophy to both serine and cysteine may be amenable to targeted therapy. Overall, our study highlights profiling of mRNA translation in cancer as an underexplored avenue for identification of previously unrecognized subtypes together with potential treatments.

PO-089 Identification of a LAMC2-regulated network featuring targetable effectors for dual therapies in pancreatic cancer. Shruthi Narayanan[1], Oihane Erice[2], Iker Feliu[2], Caterina Vicentini[3], Rodrigo Entrialgo-Cadierno[2], Karmele Valencia[2], Elisabet Guruceaga[4], Purvesh Khatri[5], Vicenzo Corbo[3], Silvestre Vicent Cambra[6], Mariano Ponz-Sarvise[1]. [1]Clinica Universidad de Navarra, Medical Oncology Department, Pamplona, Spain, [2]University of Navarra, Center for Applied Medical Research, Program in Solid Tumors, Pamplona, Spain, [3]Department of Diagnostics and Public Health, University of Verona, Verona, Italy, [4]University of Navarra, Center for Applied Medical Research, Computational Biology Program, Pamplona, Spain, [5]Stanford University, Stanford, CA, [6]University of Navarra, Center for Applied Medical Research, Program in Solid Tumors and Department of Pathology, Anatomy and Physiology; IdiSNA, Navarra Institute for Health Research; Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain, Pamplona, Spain.

Background: Pancreatic cancer stands as one of the deadliest tumors, in part due to the limited efficacy of currently existing therapies. Pancreatic cancer is characterized at the genomic level by the almost universal presence of KRAS mutations. Phenotypically, it features an incipient desmoplastic stroma with a protein component formed by collagens, fibronectin and laminins. A member of the laminin family, LAMININ γ2 or LAMC2, was previously identified by our group as part of a cross-tumors KRAS signature whose high expression was a marker of poor survival in PDAC patients. While a role for LAMC2 in migration and invasion has been previously described, little is known about a potential function in cell proliferation and viability. Moreover, LAMC2 inhibition has been shown to enhance the activity of conventional chemotherapeutic agents. However, whether its abrogation can increase the effect of targeted therapies is yet to be defined. Finally, the direct relationship between LAMC2 and the KRAS oncogene network is yet

to be defined. **Methods:** A meta-analysis of several human PDAC data sets and survival analysis of the TCGA data were performed to query the expression levels and prognosis significance of LAMC2. Human and mouse PDAC cell lines as well as a mouse model of PDAC (KrasLSLG12D, Tp53f/f, Ptf1aCre) were used to assess LAMC2 expression. In vitro (2D and 3D organoids) and in vivo models derived from human and mouse PDAC cell lines or primary tumors were used to define the functional role of LAMC2 in PDAC. Cellular and molecular analysis were deployed to dissect the mechanism of action of LAMC2 in PDAC. A dual pharmacological combination based on LAMC2-regulated effectors was also tested in human and mouse pancreatic cancer models. **Results:** At the clinical level, we observed that LAMC2 is overexpressed in human PDAC patients with regard to normal tissue. The overexpression of LAMC2 was recapitulated in human and mouse cell lines. Genetic depletion of LAMC2 had an adverse effect in 2D proliferation, clonogenic efficiency, 3D organoid growth, and PDAC-based xenograft/allograft tumor development. This deleterious effect was driven by an induction in apoptosis and a decrease in S phase consistent across species. Mechanistically, LAMC2 was linked to the KRAS pathway via transcriptional regulation of the transcription factor FOSL1/AP1, which was also a member of the cross-tumors signature. Furthermore, LAMC2 controlled a gene signature that overlaps with KRAS- and FOSL1-regulated gene signatures, which was also highly expressed in various pancreatic cancer data sets, and includes the targetable kinase AXL. Lastly, concomitant pharmacological inhibition of AXL and the FOSL1 upstream regulator MEK1/2 displayed a more adverse effect on human and pancreatic pancreatic cancer than each single drug alone. **Conclusion:** These data suggest that LAMC2 is a molecular target tightly linked to KRAS oncogene signaling that regulates downstream effectors amenable to the development of combination therapies.

PO-090 TGF-β induced EMT gene expression is associated with promoter demethylation in pancreatic cancer. Manjul Rana[1], Abul Elahi[1], Abidemi O. Ajidahun[1], Rita G. Kansal[1], Anders E. Berglund[2], David Shibata[1], Evan S. Glazer[1]. [1]UTHSC, Memphis, TN, [2]Moffitt Cancer Center, Tampa, FL.

INTRODUCTION: Transforming growth factor-β (TGF-β), a ubiquitous molecule in pancreatic ductal adenocarcinoma (PDA) tumors, acts as a potent inducer of tumor invasion by regulating proteins involved in metastasis and driving epithelial-mesenchymal transition (EMT). We hypothesized that TGF-β signaling-induced EMT is regulated by DNA methylation. To evaluate this, we investigated the association between EMT gene promoter methylation status and gene expression in the TCGA PDA dataset. We also investigated vimentin gene (a mesenchymal marker)-specific changes in DNA methylation due to TGF-β treatment in PDA cells. METHODS: The methylation status of EMT-related genes (*ZEB2*, *CDH2*, *Vimentin*) was interrogated in the TCGA PDA data set. β-values (proportion of methylated gene probes in a location) were compared to gene expression levels. Next, a PDA cell line (Panc-1) was treated with TGF-β (10ng/ml), vehicle control, DNA methyltransferase inhibitor (5-azacytidine, AZA, 10mM), or TGF-β plus AZA to evaluate the effects on the DNA methylation status of the vimentin gene with methylation-specific PCR (MSP) against the promoter region of vimentin. Unmethylation and methylation levels at the vimentin promoter region were then compared. RESULTS: We found that β values of promotor regions of *ZEB2*, *CDH2*, and *Vimentin* were moderately negatively correlated with gene expression (Pearson r varies from 0.45 to 0.63) in the PDA TCGA data set. In Panc-1 cells, vimentin gene expression was

increased following treatment with AZA compared to controls suggesting that DNA demethylation increases vimentin gene expression. MSP demonstrated that TGF-β treatment increased unmethylated vimentin gene levels compared to vehicle control treatment. TGF-β treatment increased unmethylated vimentin gene levels more so than methylated gene levels. CONCLUSIONS: These results demonstrate that gene expression of EMT-related genes may be at least partially regulated by DNA methylation. We demonstrate that TGF-β treatment leads to increased *Vimentin* promotor demethylation (lower β value) and increased gene expression in Panc-1 cells. This observation is consistent with our findings in the TCGA data set that lower β-values are associated with increased expression of mesenchymal genes, indicative of EMT. Further studies are underway to investigate the relationship between DNA methylation and TGF-β-induced EMT in general.

PO-091 Histamine receptor 1 (HRH1): A potentially novel G protein-coupled receptor (GPCR) therapeutic target in pancreatic adenocarcinoma (PDAC) cells and tumors. Cristina Salmeron, Krishna Sriram, Mehrak Javadi-Paydar, Paul A. Insel. UCSD, La Jolla, CA.

A recent study reported that patients taking HRH1 antihistamines have decreased progression of various tumors, including PDAC; the authors inferred that this was an immune effect (Fritz et al., PMID: 33550204). We have initiated studies to test an alternative hypothesis: HRH1 expressed by PDAC cells may contribute to the malignant phenotype and if so, FDA-approved HRH1 antihistamines might be therapeutics to treat or perhaps prevent PDAC. We have undertaken bioinformatic and experimental approaches to test this hypothesis. Our bioinformatic analysis revealed that PDAC tumors in The Cancer Genome Atlas (TCGA) have >30-fold higher HRH1 expression than in normal pancreas (GTEx database) and is highly expressed in PDAC cell lines in the Cancer Cell Line Encyclopedia (CCLE). HRH1 expression was selectively associated with markers of PDAC cells and not with markers of other cell types in the tumor microenvironment. Higher expression of HRH1 in TCGA-PDAC tumors negatively impacts on patient survival. Our experimental studies indicate that human and mouse PDAC cells express HRH1 mRNA, protein and signaling and that HRH1 is present on the surface of PDAC cells. We found that histamine prominently increases calcium [Ca^{2+}] in multiple human PDAC cell lines with EC_{50} values comparable to that in other cell types. The histamine-stimulated increase in [Ca^{2+}] occurs via a Gq/11 (heterotrimeric GTP binding protein)-dependent mechanism and is blocked by multiple FDA-approved HRH1 antihistamines (with pK_i values similar to those of HRH1 inhibition of other cell types). HRH1 activation by histamine increases PDAC cell migration. Histamine also increases the production of numerous cytokines (including VEGF) from PDAC cells, and in preliminary studies, stimulates growth of multiple PDAC cell lines at low concentrations (1-10 nM). Together with published data indicating that mast cells (which synthesize and release histamine) in PDAC tumors are associated with PDAC cell growth/invasion, angiogenesis and worse prognosis, our findings suggest that independent of immune cells, a "mast cell-histamine-PDAC cell HRH1 axis" may contribute to the malignant phenotype of PDAC tumors. Importantly, HRH1 on PDAC cells could be targeted by repurposing approved HRH1 antihistamines as a novel therapeutic approach for PDAC tumors. Supported by grants from the University of California Cancer Research Coordinating Committee and Tobacco-Related Disease Research Program.

PO-092 Influence of the IL-13-receptor alpha 1 chain on the malignant phenotype of pancreatic cancer cells. Jingwei Shi, Marko Kornmann, Benno Traub. University of Ulm, Ulm, Germany.

Introduction: Increasing evidence indicates salient activities of Interleukin (IL)-4, IL-13 and their specific Type-II-IL-4 receptor complex (IL-4Rα/IL-13Rα1) in carcinomas including pancreatic cancer. While IL-4 seems to be tumor-promoting in PDAC, the specific role of the IL-13/IL-13Rα1 axis was yet to be determined. Both cytokines are abundant in PDAC through cells of the tumor microenvironment. In this project, we planned to determine the specific effects of the IL-13Rα1-receptor chain on the malignant phenotype of pancreatic cancer cells. **Methods:** Expression of IL-13Rα1, IL-4Rα, and the human common gamma chain (γc) in cultured pancreatic cancer cell lines AsPC-1, Capan-1, PANC-1, MIA PaCa-2 and A818-6 as well as downstream signal transduction factors of IL13-Rα1 were detected by Western blot. The role of IL-13Rα1 in pancreatic cancer cell proliferation, mobility and migration was demonstrated based on the successful establishment of stable shRNA based IL-13Rα1-knockdown (KD) clones in Capan-1 and MIA PaCa-2 cell lines. **Results**: Not only the basal anchorage-dependent growth but also the anchorage-independent growth in soft agar were inhibited in Capan-1 and MIA PaCa-2 cells due to IL-13Rα1-downregulation. Mechanistically, IL-13Rα1-KD was associated with increased apoptosis. Additionally, signal transduction factors STAT3, STAT6, PI3K, Akt and ERK1/2 were activated in Capan-1 exposed to exogenous IL-4 and IL-13. Interestingly, IL-13Rα1-KD reduced the basal expression of STAT3, Akt and ERK1/2. IL-4 and IL-13 induced activation of STAT3, ERK1/2, Akt and STAT6 was significantly suppressed in Capan-1-IL-13Rα1-KD (C-KD) clones. Furthermore, IL-13Rα1-KD resulted in enhanced mobility and migration in Capan-1 cells. **Conclusion:** It was demonstrated that endogenous IL-13Rα1 is vital for pancreatic cancer cell growth with the involvement of STAT3/6, ERK1/2 and Akt. Surprisingly, IL-13Rα1-KD resulted in enhanced mobility and migration. Overall, IL-13Rα1 plays a critical but heterogenous role in PDAC. Our findings increase the understanding of the different functions and mechanisms involving IL-13Rα1 in pancreatic cancer progression.

PO-093 JNK2 suppresses the growth and invasion of pancreatic cancer and is opposed by JNK1. Jingwei Shi, Xiaodong Tian, Marko Kornmann, Benno Traub. University of Ulm, Ulm, Germany.

Introduction: Pancreatic cancer cells are exposed to multiple stressors with both endogenous (proliferation, metabolism and nutrient deprivation) and exogenous (medical treatment) origins. The c-Jun N-terminal protein kinases (JNKs) JNK1 and JNK2 are important stress-activated kinases, responsible for the cellular response to these stressors. Both were demonstrated to exert both tumor-suppressing and tumor-promoting effects in human cancers Most previous studies rely on nonspecific pharmacological JNK inhibition. In this study, we planned to determine the isoform-specific roles in PDAC. **Methods:** Specific JNK1- and JNK2-knockdown (KD) clones were established in cultured human pancreatic cancer cell lines MIA PaCa-2 and PANC-1 by using specific shRNA plasmids. Anchorage dependent and independent growth, as well as migration and invasion, were analyzed in JNK-1/2 KD clones and compared to pharmacological JNK inhibition using SP600125. Xenograft growth was assessed using an orthotopic mouse model. Mechanistically, we assessed changes in EMT markers both in vitro and in vivo.

Results: JNKs were expressed in both cultured pancreatic cancer cell lines as well as in resected human pancreatic cancer samples. Specific downregulation of JNK2 resulted in an overall increase in cell proliferation, invasion and alterations in cytoskeleton structure, while JNK1-KD revealed opposite effects. Nonspecific JNK-inhibition using SP600125 inhibited cell growth of all cell lines except PANC-1. **Conclusion:** JNK1 and JNK2 play opposing roles in human pancreatic cancer. Specifically targeting JNK1 with the retained function of JNK2 may provide a potential, individualized approach for targeting pancreatic cancer progression.

PO-094 Gα13 loss in KPC mouse model promotes well-differentiated pancreatic tumors that are susceptible to mTOR inhibition. Mario A. Shields, Christina Spaulding, Mahmoud G. Khalafalla, Thao N. D. Pham, Hidayatullah G. Munshi. Northwestern University, Chicago, IL.

Gα13 transduces signals from G protein-coupled receptors. Gα13 is pro-tumorigenic in epithelial cancer cell lines, which contrasts with its tumor-suppressive function in transgenic mouse models of lymphomas. We investigated the role of Gα13 in pancreatic tumor development and progression in a transgenic mouse model. Here we show that while loss of Gα13 in pancreatic cell lines decreases tumor growth *in vivo*, Gα13 loss in the Kras-driven (KC) mouse model of pancreatic tumor initiation does not affect tumor development or survival. Instead, Gα13 loss in the Kras/Tp53 (KPC) transgenic mouse model of advanced pancreatic cancer promotes well-differentiated tumors with increased tumor burden and reduced survival. Mechanistically, Gα13 loss in the KPC mouse model enhances E-cadherin-mediated cell-cell junctions and mTOR signaling. Gα13-deficient pancreatic cancer cell lines derived from these mice form well-differentiated tumors, which are susceptible to mTOR inhibition with rapamycin. Importantly, human pancreatic cancers with low Gα13 expression also exhibit increased E-cadherin protein expression and mTOR signaling. This work establishes a context-dependent role of Gα13 in pancreatic tumorigenesis, demonstrating a tumor-suppressive role in transgenic mouse models of advanced pancreatic cancer.

Tumor Microenvironment

PO-095 A cancer cell-intrinsic GOT2-PPARδ axis suppresses antitumor immunity. Jaime Abrego[1], Hannah Sanford-Crane[1], Chet Oon[1], Xu Xiao[2], Courtney Betts[1], Duanchen Sun[3], Shanthi Nagarajan[4], Zheng Xia[3], Lisa Coussens[1], Peter Tontonoz[5], Mara Sherman[1]. [1]Department of Cell, Developmental & Cancer Biology, Oregon Health & Science University, Portland, OR, [2]Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA, [3]Computational Biology Program, Oregon Health & Science University, Portland, OR, [4]Medicinal Chemistry Core, Oregon Health & Science University, Portland, OR, [5]Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA.

Glutamic-oxaloacetic transaminase 2 (GOT2) encodes a product with dual functions: primarily as a metabolic enzyme in mitochondria but also as a putative fatty acid (FA) binding protein. The purpose of this study is to investigate GOT2 function and FA-binding in PDA carcinogenesis. Depletion of GOT2 expression with CRISPR-Cas9 or shRNA in human and murine PDA cell lines shows no proliferation defects *in vitro*. However, GOT2-depleted cells orthotopically implanted in immunocompetent hosts fail to form large tumors. Analysis of cancer

cell proliferation shows no difference between control and knockout (KO) tumors, indicating proliferation is not affected by GOT2 *in vivo*. Genes negatively correlated with GOT2 expression in human PDA reveal ontology clusters associated with adaptive immune response, which suggests an immune-modulatory role of GOT2. Quantitation of immune markers using conventional and multiplex immunohistochemistry confirmed enhanced immune cell infiltration in KO tumors. Inhibition of T cells with blocking antibodies rescued the growth of GOT2 KO tumors, further validating the role of GOT2 in mediating adaptive tumor immunity. In addition to its known mitochondrial and plasma membrane localization, GOT2 expression was observed in the nuclei of PDA cells. It was hypothesized that nuclear GOT2 can deliver FA-ligand to activate PPARs (peroxisome proliferator activator receptor), a class of ligand-activated transcription factors with tumor-promoting properties; specifically, the ubiquitously expressed PPARδ. Analysis of human PDA RNA-seq data shows a significant positive correlation between GOT2 and PPARδ target genes. GOT2-dependent PPARδ activation in PDA cells was confirmed *in vitro*. Computational modeling of the crystal structure of GOT2 revealed a suitable arachidonic acid (AA) docking site, a known PPARδ ligand. Lipid binding assays of purified protein confirmed direct GOT2-AA binding. The FA docking site of GOT2 was mutated and KO cells were reconstituted with wild type and mutant GOT2. GOT2 mutant cells, compared to wild-type GOT2 cells, showed reduced PPARδ activity, nuclear localization, and interaction with PPARδ. *In vivo*, mutant GOT2 increased immune cell infiltration. Lastly, the rescue of PPARδ activity in GOT2 KO tumors restores the formation of large tumors with similar immune microenvironments to control tumors. We conclude that the enzymatic function of GOT2 is dispensable for PDA proliferation. However, GOT2 direct FA binding enhances activation of PPARδ to promote an immune-suppressed microenvironment. This non-canonical function of GOT2 can be further explored to elucidate mechanisms of immune evasion in PDA and aid in the development of efficient immunotherapies to improve disease outcomes.

PO-096 The synaptic protein Netrin G1 ligand (NGL-1) modulates tumorigenesis and immunosuppression in pancreatic cancer. Debora Barbosa Vendramini Costa[1], Ralph Francescone[1], Janusz Franco-Barraza[1], Tiffany Luong[1], Nina Steele[2], Benjamin Allen[2], Marina Pasca di Magliano[3], Charline Ogier[1], Igor Astsaturov[1], Kathy Q. Cai[1], Andres J. Klein-Szanto[1], Huamin Wang[4], Kerry Campbell[1], Edna Cukierman[1]. [1]Fox Chase Cancer Center, Philadelphia, PA, [2]Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, [3]Department of Surgery, University of Michigan, Ann Arbor, MI, [4]Department of Anatomical Pathology, Division of Pathology/Lab Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX.

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest types of cancer, with a 5-year survival of 10%. A major feature of PDAC is the presence of a dense fibrous stroma, due to the expansion of cancer associated fibroblasts (CAFs) and their extracellular matrix. This unique environment represents a challenge for therapies as it promotes immunosuppression, limits access to nutrients, and excludes or inactivates antitumor immune cells. Recently, we identified the ectopic expression of the neuronal protein Netrin G1 Ligand (NGL-1) in PDAC tissue, including its novel expression in immune cells and CAFs. However, the roles of NGL-1 in the tumor microenvironment (TME) of PDAC and in immune cell function are unknown and warranted further investigation. The contribution of NGL-1 to PDAC tumorigenesis was assessed by measuring the expression of NGL-1 in different models of PDAC and by

orthotopically injecting PDAC cells in wild type (WT) or NGL-1 full body knockout mice (KO). Using our *in vitro* 3D system we evaluated if NGL-1+ CAFs, compared to NGL-1 knockdown (KD) CAFs, produced less immunosuppressive factors and were able to rescue PDAC cell survival under nutrient deprivation. For NGL-1 dependent immune cell functions we isolated naïve immune cells from WT and KO mice and performed ex-vivo functional assays. NGL-1 expression in fibroblasts correlated with disease development in different models of PDAC, and myeloid, T and NK cells from tumor bearing mice tended to overexpress NGL-1 when compared with cells from naïve mice. Accordingly, NGL-1 KO mice orthotopically injected with PDAC cells developed smaller tumors with decreased secretion of immunosuppressive factors, increased presence of CD8+ T cells and CD4+ T cells expressing less pro-tumor markers. Single cell RNA sequencing data from tumors from KO mice showed downregulation of pro-tumor genes in different cell populations, with the fibroblastic populations differing between WT and KO mice. In order to evaluate the contribution of the immune system for tumorigenesis in WT and KO mice, we performed bone marrow chimeras and depletion of specific immune cells. Functionally, CD8+ and CD4+ T cells from KO mice proliferated more when stimulated *in vitro*, suggesting that NGL-1 could represent a functional brake for T cells, inhibiting their anti-tumor capacity. The lack of NGL-1 in stimulated bone marrow-derived macrophages decreased pro-inflammatory cytokine secretion, further suggesting a functional role for NGL-1 in myeloid cells. Of note, NGL-1 KD CAFs did not support PDAC cell survival *in vitro* and produced less immunosuppressive cytokines, which was phenocopied by the treatment with a peptide targeting NGL-1. Translationally, we assessed the overall survival of 140 PDAC patients according to NGL-1 expression in the TME, where low expression of NGL-1 in CAFs and immune cells correlated with better survival of PDAC patients. Overall, this suggests NGL-1 as potential new target in PDAC, that could be manipulated in different compartments in pancreatic cancer.

PO-097 Addition of losartan to FOLFIRINOX and chemoradiation reduces the expression of pro-invasive and immunosuppressive genes in locally-advanced pancreatic cancer. Yves Boucher[1], Jessica M. Posada[2], Sonu Subudhi[2], Ashwin S. Kumar[2], Ivy X. Chen[2], Mei R. Ng[2], Mari Mino-Kenudson[3], Nilesh Talele[2], Dan G. Duda[2], Dai Fukumura[3], Janet E. Murphy[3], Jeffrey W. Clark[3], David P. Ryan[3], Carlos Fernandez-Del Castillo[3], Theodore S. Hong[3], Rakesh K. Jain[3]. [1]Massachusetts General Hospital and Harvard Medical School, Boston, MA, [2]Massachusetts General Hospital, Boston, MA, [3]Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Purpose: A phase II trial in patients with locally-advanced pancreatic cancer (LAPC) revealed unprecedented rates of complete surgical resection after adding losartan (L) to FOLFIRINOX (FFX) chemotherapy followed by chemoradiation (CRT) (NCT01821729). The aim of this study was to identify potential mechanisms of benefit by assessing the effects of FFX-L+CRT and FFX+CRT on the stromal tumor microenvironment. **Experimental Design:** We performed a gene analysis of RNA extracted from pancreatic cancer (PC) tissue sections (NanoString *nCounter PanCancer Immune Profiling Panel* of 730 genes) and immunohistochemistry using surgical samples from patients treated with FFX+CRT (N=15), FFX-L+CRT (N=17) or underwent surgery upfront, without any neoadjuvant therapy (N=9). **Results:** In comparison to untreated PC, we found 314 and 243 differentially expressed genes (DEGs, adjusted p-value < 0.05) in FFX-L+CRT and FFX+CRT, respectively, and 54 DEGs between FFX-L+CRT and FFX+CRT. PCs from both neoadjuvant FFX-

L+CRT and FFX+CRT had increased expression of genes linked to blood vessel maturation (*CDH5, THBS1)*, transvascular migration of leukocytes (*JAM3, PECAM1, MCAM, ICAM2*), T cell activation (*CD6, ALCAM, NFATC1*), cytolytic activity of NK cells and T cells (*GZMA, GZMB, GZMH, KLRB1*) and dendritic cell (DC) related genes (*CD209, CD1C, IL3RA*). The *FOXP3* gene — encoding for a transcription factor that regulates the activity of CD4+ regulatory T cells (Tregs)— was down-regulated in FFX-L+CRT versus untreated samples. Direct comparison of FFX-L+CRT versus FFX+CRT showed increased expression of genes involved in lymphocyte activation (*NFATC4, DPP4, STAT5B*), and reduced expression of genes that regulate B cell activity (*CD22, TRAF3, MS4A1*) and *CEACAM6,* which promotes PC invasion. We also analyzed the correlation of each gene (n=730) with overall survival (OS). For patients treated with FFX-L+CRT, improved OS was negatively correlated with genes that promote invasion in PC (*PBK*), B cell development and signaling *(SYK, BLNK*), infiltration of monocytes and macrophages in tumors (*CCR2*), immune checkpoint (*BTLA*) and inhibit angiogenesis (*TNFSF15*). In patients treated with FFX+CRT, OS was positively correlated with genes that stimulate inflammation (*IL32*), T cell and DC activation (*CD48*), presentation of glycolipid antigens (*CD1E*) and antigen processing and presentation by DCs (*CD209*).Immunohistochemistry studies revealed significantly less residual disease and a higher infiltration of CD8+CD3+ T cells in FFX-L+CRT than FFX+CRT treated tumors. In addition, in patients treated with FFX-L+CRT we found significantly fewer Tregs in PC lesions with a complete/near complete versus poor/no response, confirming our transcriptomic findings. **Conclusions:** Our findings suggest that FFX-L+CRT can normalize the vasculature, and reduce invasion and the immunosuppressive effects of B cells, CCR2-positive macrophages and Tregs, and thus improve treatment outcome in patients with LAPC, although additional studies are needed.

PO-098 Longitudinal profiling of pancreatic cancer patients identifies interleukin-8 as a mediator of myeloid-epithelial crosstalk. Eileen S. Carpenter[1], Samantha Kemp[2], Padma Kadiyala[1], Nina Steele[1], Ahmed Elhossiny[1], Stephanie The[1], Valerie Gunchick[1], Rémy Nicolle[3], Michelle Anderson[1], Wenting Du[1], Carlos Espinoza[1], Richard Kwon[1], Erik-Jan Wamsteker[1], Anoop Prabhu[1], Allison Schulman[1], Vaibhav Sahai[1], Timothy Frankel[1], Filip Bednar[1], Marina Pasca di Magliano[1]. [1]University of Michigan, Ann Arbor, MI, [2]University of Pennsylvania, Philadelphia, PA, [3]Tumour Identity Card Program (CIT), French League Against Cancer, Paris, France.

Background: Pancreatic ductal adenocarcinoma (PDAC) is the 4th leading cause of cancer-related death in the US. A key hallmark of this disease is that, while tumors initially show susceptibility to standard chemotherapeutic agents, most patients eventually develop resistance, leading to poor survival. While the mechanisms of chemoresistance are unclear, murine studies have implicated the myeloid compartment of the tumor immune microenvironment. Correlative data in human tumors supports this notion, however, mechanistic studies are lacking, thus impairing translation to the clinic. The study of human pancreatic cancer has historically been challenging due to difficulty of fresh biospecimen acquisition, patient heterogeneity, and a diverse tumor microenvironment. Moreover, the vast majority of pancreatic cancer patients do not qualify for surgical resection, further limiting tissue availability. We have overcome these difficulties by developing a pipeline to analyze human tumor samples and matched blood using high-fidelity techniques including single-cell RNA sequencing (scRNAseq) and mass cytometry

(CyTOF), together with establishment of organoids from the same tumors. Notably, in this pipeline we can use small amounts of tissue from endoscopic fine needle biopsies, thus allowing us to sample tumors from patients at any disease stage. **Results:** We performed CyTOF on longitudinally-matched peripheral blood mononuclear cells (PBMCs) from 30 patients and single-cell RNA sequencing on 6 patients in the treatment naïve and on-treatment (FOLFIRINOX) state. CyTOF revealed distinct alterations in the myeloid population, with a shift toward $CXCR2^{hi}PD\text{-}L1^{hi}$ granulocytes with FOLFIRINOX treatment over time. Analysis of PBMCs from scRNAseq showed a distinct myeloid gene signature with FOLFIRINOX and in particular highlighted interleukin-8 (IL8), a chemokine involved in myeloid cell chemotaxis that is associated with poor prognosis in pancreatic cancer. Further mapping of IL8 in tumor tissue by scRNAseq showed that it is highly expressed in subpopulations of tumor epithelial cells and tumor-infiltrating granulocytes. IL8-high tumor-infiltrating granulocytes also highly expressed VEGF and CXCR4, suggesting immunosuppressive and angiogenic roles. IL8-high tumor epithelial cells were found to have a basal-like phenotype and also expressed a network of other chemokines including CXCL1, CXCL3, CXCL5, which are known to recruit immunosuppressive myeloid cells. **Conclusions:** Through longitudinal and multimodal mapping using PDAC patient blood and tumor biospecimens, we have identified IL8 as a potential mediator of epithelial-myeloid crosstalk in PDAC chemoresistance and tumor aggression. Validation studies using an all-human co-culture system of PDAC patient-derived organoids and myeloid cells are currently underway.

PO-100 Lorazepam promotes desmoplasia and ischemic necrosis in murine pancreatic ductal adenocarcinoma. Abigail C. Cornwell, Abdulrahman A. Alahmari, Arwen A. Tisdale, Kathryn Maraszek, Swati Venkat, Michael E. Feigin. Roswell Park Comprehensive Cancer Center, Buffalo, NY.

The goal of this research is to identify the effect of the GPR68-activator benzodiazepine (BZD) lorazepam on the pancreatic ductal adenocarcinoma (PDA) tumor microenvironment (TME). BZDs are commonly prescribed to PDA patients to treat anxiety and anticipatory nausea prior to chemotherapy. Certain types of BZDs are known to promote off-target activation of GPR68 under acidic conditions. We hypothesize that GPR68-activating BZDs will stimulate pro-fibrotic and pro-inflammatory signaling pathways by cancer-associated fibroblasts (CAFs), producing a more desmoplastic TME that will subsequently constrict the tumor vasculature, decreasing chemotherapeutic efficacy, and ultimately decreasing patient survival. Using a subcutaneous KPC allograft mouse model, we found that lorazepam modified the TME by significantly increasing α-SMA (smooth muscle actin) protein expression, collagen deposition, and ischemic necrosis. Preliminary, we found that treating tumor-bearing KPC mice with lorazepam similarly promoted ischemic necrosis. RNA sequencing of the lorazepam-treated allograft tumors indicated that CAF and ECM-related genes such as PDPN, LOX, SERPINB2, and ITGA11 were significantly upregulated. Pathway analysis revealed that lorazepam treatment promoted pathways related to inflammation, EMT, hypoxia, as well as known GPR68 downstream signaling pathways such as TNF-alpha signaling via NF-kB and IL6/JAK/STAT3 signaling. qRT-PCR of immortalized CAFs treated with lorazepam indicated that IL6 expression is increased by lorazepam in a GPR68-dependent manner at acidic pH, supporting that the promotion of IL6 expression we observed in vivo was likely GPR68 and CAF-dependent. To validate the clinical significance of our work, covariate adjusted analyses of pancreatic cancer

patients who received chemotherapy at Roswell Park from 2004-2020 was performed. Patients taking GPR68 activator benzodiazepines versus non-activator benzodiazepines had poorer progression-free survival (HR 6.85(2.12,22.06)), suggesting that GPR68 activation by benzodiazepines may negatively impact survival. Overall, these findings suggest that lorazepam significantly modifies the PDA TME by promoting desmoplasia and ischemic necrosis, due in part to activation of GPR68. Future experimental work will determine if lorazepam negatively impacts survival and chemotherapeutic efficacy. Significance: This research may guide the development of new clinical recommendations for prescribing benzodiazepines to PDA patients, which will likely be applicable to other cancer types.

PO-101 Characterization of longitudinally collected fine needle aspiration biopsies of pancreatic ductal adenocarcinoma upon endoscopic ultrasound guided radiofrequency ablation. Krisha Desai[1], Patrick Varun Lawrence[1], Christopher Wadsworth[2], Nagina Mangal[2], Nagy Habib[2], Anguraj Sadanandam[1], Mikael Sodergren[2]. [1]Institute of Cancer Research, London, United Kingdom, [2]Imperial College, London, United Kingdom.

Background: Most patients with pancreatic ductal adenocarcinoma (PDAC) are metastatic at presentation with dismal prognosis warranting improved systemic therapy options. Longitudinal sampling for assessment of treatment response poses a challenge for validating novel therapies. In this proof-of-principle study, we evaluate the role of endoscopic ultrasound (EUS)-guided serial fine-needle aspiration biopsies (FNABs) to study the mechanism of action of radiofrequency ablation (RFA). **Methods:** Patients with stage III inoperable PDAC with prior exposure to gemcitabine were selected into ARDEO (ethically approved Phase-II prospective clinical study of EUS-RFA). Post examination, targeted RF was delivered thrice and sequential FNABs of tumor were taken before and after treatment. Transcriptomic profiling of 6 FNABs from 2 patients was performed using a custom NanoString gene panel (144 genes) consisting of cancer and cancer-associated fibroblast (CAFs) subtypes along with immune changes. CAF culture was established from one FNAB and characterised by immunofluorescence and immunoblotting. **Results:** RFA treatments were well tolerated without any complications and both patients had stable disease immediately after EUS-RFA. Two-course RFA led to upregulation of *CD1E* gene (participates in antigen presentation) in both, patient 1 and 2 (4.5 and 3.9-fold) compared to baseline. Patient 1 showed increased expression of T cell genes (*CD4* – 8.7-fold, *CD8* – 35.7-fold), cytolytic function (6.4-fold) and inflammatory response (8-fold) post-RFA. Greater than 2-fold upregulation of *CD274 (PDL1*), *IDO1*, *PDCD1* and *TNFRSF18 (GITR)* was observed post 2nd RFA in both the patients. Further, two-course RFA led to increased *PDGFRa* (4.5 and 9-fold) in both patients along with enrichment of pCAF subtypes B and C in patient 1 and subtypes A, B and D in patient 2. Immunofluorescence staining revealed expression of PDGFRa, aSMA, VIM, POSTN and MYH11 on patient2-derived CAFs post 1st RFA; validated by immunoblotting. Finally, RFA led to downregulation of classical PDA subtype in both patients. **Conclusions:** This feasibility study validates longitudinal sampling by EUS-FNABs as an appropriate research tool to study tumor microenvironmental changes associated with local pancreatic immunomodulatory techniques like RFA.

PO-102 Fibroblast-derived interleukin-33 promotes pancreatic ductal adenocarcinoma as a result of tumor cell KRAS^{G12D}. Katelyn Donahue, Wenting Du, Carlos Espinoza, Eileen Carpenter, Kristee Brown, Nina Steele, Marina Pasca di Magliano. University of Michigan, Ann

Arbor, MI.

Pancreatic ductal adenocarcinoma (PDA) is a deadly disease that is difficult to detect early and limited in its therapeutic options. As a result, the five-year survival rate of PDA is only 10%. Expression of the oncogene KRAS is present in over 94% of PDA cases, with the most common KRAS mutation being $KRAS^{G12D}$. Another hallmark of PDA is its reactive, fibroinflammatory tumor microenvironment, which is established in tandem with the earliest stages of tumorigenesis and is essential to the growth and maintenance of the tumor. In this work, we have endeavored to uncover the ways by which tumor cell $KRAS^{G12D}$ expression influences extracellular signals that shape the tumor microenvironment to the benefit of the disease, with the overall goal of identifying new potential therapeutic vulnerabilities downstream of KRAS signaling. Importantly, using genetically engineered mouse models (GEMMs) and *in vitro* assays by which tumor cell expression of $Kras^{G12D}$ can be turned on and off, we have found that tumor cell $KRAS^{G12D}$ activity stimulates the expression of Interleukin-33 (IL33) in pancreatic fibroblasts through a mechanism requiring both JAK/STAT3 and Focal Adhesion Kinase (FAK) signaling. Through immunohistochemical staining and single cell RNA sequencing of patient tumor samples, we have also confirmed the expression of IL33 in the cancer-associated fibroblast compartment of human PDA. IL33 is a dual function cytokine - it is sequestered in the nucleus where it can impact the cellular transcriptome, and it can also be released to signal to cells expressing its receptor, ST2L. Across the cancer biology field, IL33 expression has been reported to be regulated by different signaling factors, and its impact can be either tumor promoting or tumor restricting depending on the tissue context. In PDA, the role of IL33 is currently unclear, with recent publications alternatively describing it as anti- or pro-tumor. While previous studies have focused on epithelial IL33, we have used a $Pdgfra\text{-}CreER^{T2/+};Il33^{f/f}$ GEMM to knock out Il33 in fibroblasts prior to orthotopic implantation of syngeneic PDA cells. Tumor growth in Il33-deficient mice was reduced, and we observed fewer infiltrating immune cells, including macrophages. As our group and others have previously shown that macrophages are a significant driver of immunosuppression required for the maintenance of PDA, we hypothesized that fibroblast-derived Il33 promotes pancreatic tumorigenesis through either direct or indirect recruitment of immunosuppressive macrophages. As we continue to dissect the role of IL33 in the pancreatic tumor microenvironment, we aim to fully understand the biological role of IL33 during the onset of carcinogenesis and in advanced disease. Overall, this work will shed new light on the ways by which fibroblasts and macrophages are co-opted by tumor cells as a result of $KRAS^{G12D}$, further elucidating prospective therapeutic avenues that may be exploited in the future.

PO-103 Cellular origin influences immune microenvironment in a pancreatic cancer mouse model with loss of Pten and activation of Kras. Yan Dou[1], Wesley Hunt[1], Justin Chhuor[1], Farnaz Taghizadeh[1], Atefeh Samani[1], Karnjit Sarai[1], Claire Dubois[2], David F. Schaeffer[1], Maike Sander[2], Janel L. Kopp[1]. [1]University of British Columbia, Vancouver, BC, Canada, [2]University of California-San Diego, La Jolla, CA.

Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease with an overall 5-year survival rate of merely 9%. Although mouse studies in the past decade have made progress towards a better understanding of how PDAC cellular origin affects tumorigenesis, there hasn't been study on the immune microenvironment differences between precursor lesions and PDAC

derived from acinar and ductal cells. Following our previous study that showed loss of *Pten* with oncogenic *Kras* ^{G12D} mutations in the ductal cells (*KPten*$^{Duct/+}$) resulted the formation of intraductal papillary mucinous neoplasias (IPMN) as the precursor lesion in mice, we further found siminar mutations in the acinar cells (*KPten*$^{Acinar/+}$) formed pancreatic intraepithelial neoplasia (PanIN) instead. We subsequently used the *KPten*$^{Duct/+}$ and *KPten*$^{Acinar/+}$ models to elucidate the effect of cellular origin on the immune microenvironment by performing immunohistochemistry. We looked at immune cell infiltration densities between precursor lesions and PDAC derived from *KPten*$^{Duct/+}$ and *KPten*$^{Acinar/+}$ models and will present the data during the conference. Additionally, macrophages polarized by conditioned media derived from *KPten*$^{Duct/+}$ and *KPten*$^{Acinar/+}$ PDAC cells showed distinctive polarization status, indicating cellular origin could result in PDAC with different cytokine and chemokine profiles that affect the immune microenvironment. Our study is the first to directly compare immune cell population between acinar- and ductal-derived PDAC originating from different types of precursor lesions with the same genetic background. Our study suggests the potential role of cellular origin on influencing PDAC immune heterogeneity.

PO-104 Activation of WNT signaling in CD4$^+$ T cells promotes immune suppression in pancreatic cancer. Wenting Du, Rosa E. Menjivar, Katelyn Donahue, Ashley Velez-Delgado, Marina Pasca di Magliano. University of Michigan, Ann Arbor, MI.

WNT ligand expression and activation of the WNT signaling have been associated with pancreatic ductal adenocarcinoma (PDA). Using a mouse model that recapitulates the stepwise progression of PDA, we previously showed that epithelial WNT signaling is required for PDA initiation and progression. Through single cell RNA sequencing, we discovered that, in addition to epithelial cells, infiltrating immune cells and in particular T cells express the WNT signaling machinery, including receptors, downstream effectors and target genes. In particular, T cells express high levels of *Tcf7*, encoding the transcription factor TCF1. TCF1 is important for T cell development, but its role in adult T cells and specifically in the setting of pancreatic cancer remained unknown. To address this, we generated mice that allow deletion of *Tcf7* specifically in CD4$^+$ T cells in an inducible manner. We then implanted orthotopic syngeneic tumors in control mice or in mice lacking *Tcf7* in CD4$^+$ T cells, and observed reduced tumor growth in the latter. Analysis of the tumors revealed an increase in apoptotic cells; while the prevalence of infiltrating T cells did not change, we observed an increase in cytotoxic marker expression, such as Granzyme B, consistent with increased T cell activation. We observed decreased regulatory T cells, possibly indicating less immune suppression. However, we also observed a compensatory infiltration of increased granulocytic myeloid derived suppressor cells. Deletion of *Tcf7* in CD4$^+$ T cells also re-polarized the immunosuppressive macrophages into pro-inflammatory macrophages, and alterations of inflammatory cytokines secreted by cancer associated fibroblasts. Taken together, this study reveals an important role of WNT signaling in CD4$^+$ T cells in the PDA microenvironment. We are currently evaluating ways to exploit changes in the immune microenvironment upon inactivation of *Tcf7* in CD4$^+$ T cells in combination therapy approaches.

PO-105 Overcoming stromal barriers in PDA with a novel polymeric Toll-like receptor agonist. Christopher C. DuFort[1], Ciana L. Lopez[2], Martin C. Whittle[1], Vladimir Vlaskin[2], Aditi Vadodkar[1], Selvi Srinivasan[2], Patrick S. Stayton[2], Sunil R. Hingorani[1]. [1]Fred Hutchinson Cancer

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The complex tumor microenvironment (TME) of PDA creates a uniquely immune- and drug-privileged sanctuary that contributes to disease pathogenesis and treatment resistance. The profoundly immunosuppressive microenvironment of PDA includes abundant MDSC, Treg, M2-like TAM and a dearth of cytotoxic T-cells. Recent discoveries of highly expressed Toll-like receptors 7 and 8 (TLR7/8) in the desmoplastic stroma of PDA suggest that TLR7/8 agonists may be an effective immune-targeted therapy. TLR7/8 agonists may inhibit tumor growth by any of several mechanisms including myeloid cell reeducation, depletion of MDSC, and/or depletion of Treg, thereby activating cytotoxic CD8+ T cells. However, the circulatory half-life of most TLR7/8 agonists is relatively short, and elevated systemic levels and prolonged treatment can also cause severe adverse reactions that can limit their use and effectiveness. To address these concerns, we have developed a novel polymeric form of a TLR7/8 agonist that exploits a breakthrough synthetic technology to directly polymerize drugs into nanocarriers with defined architectures. These agents are designed to minimize adverse effects and increase efficacy through more favorable pharmacokinetics and enhanced delivery to desmoplastic tumors with release profiles tailorable over the range of days to weeks. This agent is in development in our murine preclinical trials program (MCTP) which is modeled on human clinical trials and involves randomized, blinded, placebo-controlled studies against the current standard-of-care using the KrasLSL-G12D/+;Trp53LSL-R172H/+;p48Cre/+ (KPC) model as our primary platform of the autochthonous disease. Exploratory experiments were performed to determine optimal dose and treatment schedule. KPC mice with ultrasound-imaging documented disease meeting enrollment criteria were subsequently randomized into one of two arms in 28-day pilot studies: control vehicle-treated and polymeric TLR7/8 (n=5-8 animals per arm). Daily health and behavior checks, serial body weight measurements, and complete blood count (CBC) profiles over the course of the study revealed no adverse health effects or overt toxicities. Mice were euthanized at study completion and all organs harvested for histological analyses; single cell suspensions from blood, spleen, peri-pancreatic lymph nodes and primary tumor were analyzed by FACS and/or CyTOF Helios cytometry (incorporating 30+ specific markers and with a particular focus on identifying specific states and subtypes of CD8+ T cells as well as markers of checkpoint activation to assess innate and adaptive immunity in treated versus untreated and tumor versus normal tissues). Preliminary analyses reveal response associated with increases in T cell activation markers and concomitant changes in inhibitory markers. M1 and M2 macrophage populations also appeared to be profoundly affected, suggesting targeting of specific immune cell types and distinct activation states.

PO-106 The extrinsic and modulatory effects of CSF-1/CSF-1R signaling in generating an immunosuppressive pancreatic cancer tumor microenvironment and promoting metastasis. Gizem Efe[1], Kensuke Suzuki[1], Jason R. Pitarresi[2], Anna M. Chiarella[1], Alina L. Li[1], Anil K. Rustgi[1]. [1]Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY, [2]Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA.

Introduction: The multistep process of the tumor cell invasion-metastasis cascade, which involves the spread of cancer cells from primary tumor sites to colonization into distant organs, is a major barrier to effective therapy. Both intrinsic factors such as genomic instability and epigenetic alterations and extrinsic factors, such as microenvironmental cues, have been

implicated in contributing to the metastatic proclivity of cancer cells. The crosstalk between tumor cells and cells within the heterogenous tumor microenvironment (TME) is a critical driver of tumorigenesis. This can nurture tumor cell migration and invasion into the stroma, providing a foundation for eventual metastasis. Using our mouse models of PDAC, we have identified *Colony stimulating factor 1* (*Csf-1*; also known as *M-Csf*) to be differentially and significantly upregulated. CSF-1 is secreted by tumor cells to recruit and polarize macrophages into M2-like tumor associated macrophage (TAM) phenotype through binding to its cognate tyrosine kinase receptor colony-stimulating factor 1 receptor (CSF-1R). However, the role and mechanisms of CSF-1/CSF-1R pathway in modulating other elements of the PDAC TME that contributes to invasion and metastasis has yet to be investigated. **Methods:** We use *Pdx1-Cre; LSL-Kras$^{G12D/+}$; LSL-Trp53^{R172H}; Rosa26$^{LSL-YFP}$* (KPCY) mice and 2D/3D cell culture systems. We overexpressed *Csf-1* and established a CRISPR system to knockout *Csf-1* in our cells using ribonucleoprotein (RNP) Cas9/gRNA complex via nucleofection. These engineered cells are used to model syngeneic primary and metastatic tumor formation. We have established a novel quantitative multiplex immunofluorescence (qmIF) staining approach to characterize changes in the TME upon modulating *Csf-1* expression, specifically focusing on macrophages, myeloid-derived suppressor cell (MDSC), T-cell, B-cell, fibroblast and nerve fiber markers. **Results:** Our data from *in vivo* studies suggested that *Csf-1* overexpression leads to an increase in primary tumor growth and metastasis, while the depletion of *Csf-1* reduces metastatic burden. The automated quantification and analysis of our unbiased IF approaches has yielded the following: CSF-1 overexpression leads to increased tumor infiltration of F4/80$^+$CD163$^+$CD206$^+$ M2-polarized macrophages and decreased number of CD3$^+$CD8$^+$ cytotoxic T-cells, generating an immunosuppressive TME. Furthermore, our preliminary data demonstrated that *Csf-1* is also upregulated in cancer associated fibroblasts (CAFs), which potentially synergizes with the epithelial compartment to attract immunosuppressive immune cells and promote immune evasion. Finally, the TCGA data reveals that metastatic PDACs have increased *Csf-1* expression compared to primary tumors and overexpression of *Csf-1* is associated with reduced survival. **Conclusion:** We have demonstrated a novel role of CSF-1 upregulation in reprogramming the TME in PDAC and fostering increased metastatic capacity. We believe this can be exploited therapeutically.

PO-107 Fibroblast differentiation trajectories elicit regional tissue states in pancreatic cancer. Barbara T. Grünwald[1], Curtis McCloskey[1], Antoine Devisme[2], Foram Vyas[1], Geoffroy Andrieux[2], Kazeera Aliar[1], Faiyaz Notta[3], Grainne O'Kane[1], Julie Wilson[3], Jennifer Knox[1], Sandra Fischer[4], Thomas Kislinger[1], Melanie Boerries[2], Steven Gallinger[3], Rama Khokha[1]. [1]Princess Margaret Cancer Centre, Toronto, ON, Canada, [2]University of Freiburg, Freiburg, Germany, [3]Ontario Institute for Cancer Research, Toronto, ON, Canada, [4]University Health Network, Toronto, ON, Canada.

Intratumoral heterogeneity is a critical frontier in understanding how the tumor microenvironment (TME) propels malignant progression. We recently deconvoluted regional heterogeneity in the human PDAC stroma to assess its role in disease progression and discovered two types of 'sub-tumor microenvironments' (subTMEs), called 'reactive' and 'deserted'. These histologically definable tissue states exhibit strong regional relationships with tumor immunity, subtypes, differentiation, and treatment response. Here, we set out to define their cell biological underpinnings through a combination of subTME-specific cancer-associated fibroblast (CAF)

models, integrative histopathology, quantitative image analysis, multiOMICs, scRNAseq, and controlled functional assays. Remarkably, the growth patterns of CAF cultures closely recapitulated the characteristic histomorphology of their originating subTMEs, and these distinct phenotypes were accompanied by behavioral differences. Unsupervised graph-based clustering of scRNAseq profiles showed that CAFs largely grouped by their originating subTME yet comprised up to 10 individual clusters. The subTME-specific multi-subpopulation CAF communities self-organized into distinct 'coordinated states', represented by cluster-overarching functional profiles and distinct morpho-histological and behavioral phenotypes. These differences originated in cellular differentiation trajectories, with an 'intermediate' transitory state evident both in single cell transcriptomics and *in situ*. Noticeably, this CAF differentiation potential was associated with distinct tumor-related functions and, similar to stem cells, was marked by RNA diversity and pluripotency markers. Therefore, regional TME programs in PDAC appear to result largely from transitions between subpopulation-overarching fibroblast differentiation states that guide multifaceted CAF and immune cell communities into recurrent tissue self-organizational units.

PO-108 Evaluation of antitumor activity of modified-gemcitabine solid-lipid nanoparticle in pancreatic pdx models. Edward Agyare[1], Taylor Smith[2], Andriana Inkoom[1], Bo Han[3], Jose Trevino[4], Nkafu Bechem Ndemazie[1]. [1]College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL, [2]Food and Drug Administration, Silver Spring, MD, [3]Keck School of Medicine, University of Southern California, Los Angeles, CA, [4]Department of Surgery, College of Medicine, Virginia Commonwealth University, Richmond, VA.

Purpose: Pancreatic cancer (PCa) is one of the most aggressive and devastating malignancies in the United States, with a high mortality rate. Despite the remarkable advances in cancer therapy, there are limited treatment options for PCa patients. Gemcitabine (Gem), a nucleoside analog, remains the preferred choice, either alone or in combination with other chemotherapeutic agents, to treat PCa. While Gem has proven to be effective against PCa in the early stages of treatment, PCa cells resistance to Gem and rapid metabolism of Gem has resulted in poor treatment outcomes. The objective of this study was to address the issues associated with Gem via chemical modification of Gem to 4NSG. This significantly reduced the metabolism and highly sensitized PCa cells to Gem. **Methods:** Gem was modified by linking the 4-amino group of Gem and stearoyl acyl derivative to form 4NSG and characterized using nuclear magnetic resonance (NMR) for) to determine new bond formation, micro-elemental analysis micro-elemental analysis used to ascertain the presence of elemental composition and purity, high liquid chromatography (HPLC) used to determine percent purity of 4NSG. The 4NSG was developed into solid lipid nanoparticles (4NSG-SLN), and the size was determined using a particle size analyzer. Patient-derived primary pancreatic cancer cells (CMZ and G68Ca) and MiaPaCa-2 cells were treated with free Gem and 4NSG-SLN. Percent cell viability was determined using resazurin assay and antitumor efficacy testing of Gem, and 4NSG-SLN was performed in patient-derived xenograft (PDX) mouse models bearing G68Ca PCa. **Results:** Analysis of the H-NMR spectra displayed an amide bond at 11ppm, confirming the conjugated bond between the 4-amino group of Gem and stearoyl derivative. The half-maximal inhibitory concentration (IC_{50} =11±1.3μM) of 4NSG-SLN-treated CMZ culture was significantly higher than that of Gem treated CMZ culture (IC_{50} = 56±2 μM, $p<0.001$). We found a similar trend of higher growth

inhibition of 4NSG-SLN treated G68Ca and MiaPaCa-2 cultures ($IC_{50\ (4NSG\text{-}SLN\text{-}G68Ca)}$ = 12±2.1 µM; $IC_{50(Gem\text{-}MiaPaCa\text{-}2)}$ = 27±4 µM) respectively. Where $p< 0.001$ ($_{4NSG\text{-}SLN\text{-}G68Ca\ vs.\ Gem\text{-}G68Ca}$) and $p<0.001$ ($_{4NSG\text{-}SLN\text{-}MiaPaCa\text{-}2\ vs\ Gem\text{-}MiaPaCa\text{-}2}$) compared with Gem treated G68Ca and Mia-PaCa-2 cultures ($IC_{50\ (G68Ca)}$ = 68±26 µM; $IC_{50\ (Mia\text{-}PaCa\text{-}2)}$ =54±5 µM) respectively. Put together, the anticancer activity of 4NSG-SLN demonstrated enhanced efficacy in CMZ, G68Ca, and MiaPaCa-2 treated cultures compared with their corresponding Gem treated cultures. For antitumor efficacy testing, 4NSG-SLN demonstrated significant tumor growth inhibition in PDX models harboring G68Ca pancreatic tumors compared with Gem treated with PDX models. Immunohistostaining studies of 4NSG-SLN treated pancreatic tumors significantly reduced vascular endothelial growth factor receptor (VEGFR) expression compared with Gem treated pancreatic tumors. **Conclusion:** This study demonstrated that 4NSG might be a novel approach to significantly enhance the therapeutic efficacy of Gem in the treatment of PCa.

PO-110 Targeting cathepsin B in the pancreatic stellate cells stimulates CD8+ T cell dependent anti-tumor immune response. Bharti Garg, Tejeshwar Jain, Utpreksha Vaish, Vikas Dudeja. University of Alabama at Birmingham, Birmingham, AL.

Background: Cathepsin B (CTSB) is a lysosomal cysteine protease which has been described to have a role in pancreatic cancer development. However, its significance in the tumor microenvironment has never been investigated. Pancreatic stellate cells (PSCs) are one of the main sources of Cancer Associated Fibroblasts (CAFs) in the pancreatic cancer microenvironment. Herein, we describe the effects of targeting CTSB in the pancreatic stellate cells (PSCs) in pancreatic cancer (PDAC). **Methods:** To specifically study the role of CTSB in PSCs, PSCs were isolated from C57BL/6J (WT) or CTSB -/- mice and were orthotopically injected with KPC cancer cells into the tail of pancreas of 6-week old female WT mice. Tumors were analyzed at endpoint using flow cytometry. To further study the dependence of effects of CTSB in PSCs on adaptive immune cells, WT or CTSB -/- PSCs were co-injected with KPC cells into Rag 1-/- mice or CD8+ T cell depleted mice (anti-CD8 mAb, 250ug/dose every 3 days). *In vitro,* WT or CTSB-/- PSCs were co-cultured with KPC across a transwell. CD8+ T cells, isolated from KPC tumor bearing mice, were exposed to conditioned media from this co-culture *ex-vivo* and their cytotoxicity was measured using calcein release assay. In another experiment, WT mice injected with KPC cancer cells were treated with vehicle control or the CTSB inhibitor, CA-074 methyl ester (10 mg/kg), through daily intraperitoneal injections. **Results:** Co-injections of WT PSC with KPC cells formed larger tumors as compared to KPC cancer cells alone, however, co-injection with CTSB-/- PSCs abrogated this tumor promoting effect. Flow cytometric analysis revealed increased infiltration of Ifn-γ secreting CD8+ T cells in the KPC+CB-/- PSC group as compared to KPC+WT PSC group. Tumor decreasing effect of CTSB-/- PSCs was lost when co-injections were performed in Rag 1-/- mice or CD8+ T cell depleted mice, indicating that this effect relies on a functional adaptive immune response, specifically CD8+ T cells. *In vitro,* co-culture of CTSB-/- PSCs with KPC led to reduced expression of inflammatory CAF (iCAF) markers like IL-6 and IL-11 when compared to co-culture of WT PSCs with KPC. CD8+ T cells exposed to conditioned media from CTSB-/- PSC co-culture showed increased cytotoxicity against KPC cells *ex-vivo*, as compared to those exposed to conditioned media from WT PSC co-culture. Finally, treatment with CA-074 was able to decrease orthotopic PDAC tumor growth with increased CD8+ T cell infiltration evident on flow cytometry. **Conclusion:** Targeting CTSB in the PSCs stimulates anti-tumor adaptive

immune response against pancreatic cancer by inhibiting conversion of PSCs into the tumor-promoting iCAF phenotype. CTSB might emerge as a novel target for CAF-directed therapy in pancreatic cancer.

PO-111 A human single-cell RNA sequencing atlas of pancreatic ductal adenocarcinoma enables harmonized cell type calling and comprehensive analyses of potential intercellular signaling. Benedict Kinny-Köster[1], Melissa R. Lyman[2], Dimitrios N. Sidiropoulos[2], Melanie Loth[2], Alexandra B. Puscek[2], Laura D. Wood[3], Jin He[1], Jun Yu[1], Richard A. Burkhart[1], Elizabeth M. Jaffee[2], Jacquelyn W. Zimmerman[2], Elana J. Fertig[2]. [1]Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, MD, [2]Department of Oncology, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD, [3]Department of Pathology, Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins University School of Medicine, Baltimore, MD.

Purpose: Pancreatic ductal adenocarcinoma (PDAC) is characterized by a dismal prognosis, low ductal cancer cellularity and a dominant tumor microenvironment (TME) in response to malignant degeneration. Modern single-cell RNA sequencing (scRNA-seq) platforms fundamentally improved the opportunities to analyze PDAC biology through isolation of diverse cell types including ductal, mesenchymal, myeloid and lymphoid populations. Published scRNA-seq data of PDAC patients provide innovative and astounding insights, but are limited by cohort size and intrinsically vulnerable to internal biases. Herein, we present a human single-cell PDAC atlas which links the previous sequencing efforts aiming for increasing depth and robustness with the combined analysis of transcriptomes. **Methods:** We selected scRNA-seq data of all patients with PDAC from six publicly available datasets (published between 2019-2020). Altogether, 61 different human samples with 142,807 cells were integrated into one dataset leveraging 15,219 genes, which were conclusively identical between all datasets based on the utilized nomenclature in the provided raw data. In addition, we extracted 16 samples with 31,587 cells from control pancreas specimens which were included in three out of the six datasets. The analyses were performed using the R statistics and Python environments utilizing established software including Monocle3 and Seurat. **Results:** After computational preprocessing of the integrated dataset, cell types were identified based on differentially expressed and canonical markers. The generated PDAC atlas consists of 26% ductal cancer, 2% ductal normal, 12% mesenchymal (stellate cells and cancer-associated fibroblasts), 18% myeloid, 19% lymphoid and 23% other cells (including acinar and endocrine cells). Copy number variation analyses confirmed the discrimination between cancer and normal ductal cells. Certain subpopulations within cell types were mapped based on the expression of supervised gene sets. Within the ductal cancer cell population, the Classical and Basal-like Moffitt signatures coexisted in the majority of patients with distinct ratios and predominance, which were associated with differences in the TME composition. Furthermore, the presence of myofibroblasts and inflammatory fibroblasts could be quantified at the patient-level. The reconstruction of intercellular signaling between ductal cancer cells and several TME components revealed potential ligands, receptors and transcription factors that may functionally define routes and polarity of cross-talk in PDAC. **Conclusion:** This human scRNA-seq atlas is the largest available dataset of patients with PDAC while harmonizing previously published data. It is engineered to analyze current knowledge gaps with increased rigor and, most importantly, overcomes obstacles related to bulk transcriptome sequencing data. Molecular characteristics of the ductal cancer cells and TME components inferred from the

presented framework are promising to identify disease- and patient-specific signaling, key regulators, and therapeutic targets.

PO-112 Stromal reprogramming by FAK inhibition overcomes radiation resistance to allow for immune priming and response to checkpoint blockade. Varintra E. Lander, Jad I. Belle, Brett L. Knolhoff, John M. Herndon, Cedric Mpoy, Buck E. Rogers, Julie K. Schwarz, David G. DeNardo. Washington University in St. Louis, St. Louis, MO.

Pancreatic Ductal Adenocarcinoma (PDAC) is one of the most lethal malignancies. While checkpoint immunotherapies are effective therapies in many solid malignancies, these same regimens have not been effective in PDAC. Furthermore, clinical trials combining checkpoint immunotherapies with standard of care chemotherapy or radiation therapy (RT), which should be able to prime anti-tumor immunity and unlock immunotherapies, have not been successful. Thus, understanding why the combinations of RT and immunotherapy fail in PDAC is critical. To better understand why RT and checkpoint immunotherapies fail, we studied the impact of stereotactic body radiotherapy (SBRT), an RT regimen which delivers precise and intense doses of radiation into tumor cells, on antigen specific T cell responses in both human PDAC tissues and genetically engineered mouse models of PDAC. In human PDAC tumors, we found no increase in the number of CD8 tumor infiltrating T cells in the tumor stroma compared to a control group, which gives us no evidence of T cell priming following SBRT. Using the p48-Cre/LSL-$Kras^{G12D}$/$p53^{Flox/Flox}$/OVA-GFP^{+} (KPC-OG) mice, RT alone, despite inducing temporary tumor control did not prime new antigen specific T cell responses, similar to what we found in the human PDAC tissues. We postulated that the unique PDAC tumor microenvironment (TME), which is characterized by a fibrotic desmoplastic stroma, might play a role in limiting immune priming by SBRT. To study the role of PDAC's TME to RT response, we developed a 3D organoid *in vitro* co-culture system. We found that fibroblasts and collagen work synergistically to cause RT resistance, which is mediated in part through the hyperactivation of Focal Adhesion Kinase (FAK). In KPC mice, FAK inhibitor (FAKi) rescues RT resistance leading to significant tumor regression and enhances long-term survival. Associated with this regression, we found enhanced anti-tumor immunity in the form of increased conventional dendritic cells and tumor specific CD8 T cells. Single cell RNA sequencing data revealed that this treatment combination enhances antigen processing and presentation and T cell activation in the immune myeloid compartment and alters the composition of cancer associated fibroblasts in the PDAC stroma. Based on these data, we initiated a phase Ib study in which FAKi (VS-6063) will be given in combination with SBRT to patients with locally advanced PDAC (NCT04331041). This trial is currently underway. With this human trial underway, we next hypothesized the combination of RT and FAKi would render immunotherapy effective. Pre-clinical studies in mouse PDAC models showed that while RT and checkpoint blockade was ineffective at tumor control, the triple combination of FAKi, RT, and checkpoint blockade led to extended long-term survival. Overall, these data suggest that stromal modulation can be used to allow RT to prime anti-tumor immunity in PDAC and unlock checkpoint immunotherapy efficacy.

PO-113 The prolyl isomerase PIN1 plays a critical role in fibroblast differentiation states to support pancreatic cancer. Ellen M. Langer[1], Isabel A. English[1], Vidhi Shah[1], Kevin MacPherson[1], Kayleigh M. Kresse[1], Brittany L. Allen-Petersen[2], Colin J. Daniel[1], Mara H.

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PIN1 is a phosphorylation-directed prolyl isomerase that alters the conformation and, therefore, the function of many proteins. PIN1 overexpression in cancer contributes to cancer cell-intrinsic phenotypes including cellular proliferation and migration. While its pro-tumor functions have generated interest in therapeutic targeting of PIN1 for cancer treatment, the effects of PIN1 inhibition on tumor-associated stromal phenotypes have not yet been studied. We assessed pancreatic cancer xenografts and genetically engineered p48-*Cre*; LSL-$Kras^{G12D}$; $p53^{R172H}$ (KPC) mice that were treated with small molecule PIN1 inhibitors or crossed into a full body PIN1 knockout ($Pin1^{-/-}$), and found that PIN1 inhibition or loss decreased tumor growth and extended overall survival. To interrogate a direct role for PIN1 in the stroma, we orthotopically injected a KPC cell line into syngeneic $Pin1^{+/+}$ or $Pin1^{-/-}$ hosts and found dramatic reduction of tumor cell growth in $Pin1^{-/-}$ hosts. Further analysis of the $Pin1^{-/-}$ tumor microenvironment revealed decreased expression of alpha-SMA, a marker of myofibroblastic cancer associated fibroblasts (myCAFs), as well as decreased ECM deposition and/or organization. Pancreatic stellate cells (PSCs) activated in the tumor microenvironment play a major role in the deposition of ECM and secrete growth factors to support tumor cell proliferation and survival. We, therefore, interrogated the role of PIN1 in PSCs. We found that loss of PIN1 in PSCs inhibits TGF-beta-induced stellate cell activation into a myofibroblast phenotype. Single cell ATAC-seq analysis demonstrated that a subset of TGF-beta responsive changes to chromatin accessibility are impaired in the absence of PIN1, and suggests that specific transcription factor families may play a role in the PIN1-dependent response to TGF-beta. Further analysis of PSCs or CAFs with PIN1 loss indicated that, at baseline, these cells express gene programs consistent with the recently described antigen presenting CAFs (apCAFs). Finally, in addition to changes in cellular state and plasticity, we found that loss of PIN1 alters PSC secretion of paracrine factors that support oncogenic phenotypes. For example, PSCs with loss of PIN1 have reduced expression of HGF and increased expression of VEGF, resulting in altered cancer cell and vascular phenotypes. This work establishes a role for PIN1 in regulating fibroblast function and suggests that targeting PIN1 in cancer will have a broad anti-tumor effect. Our ongoing work continues to use 2D co-cultures, heterotypic 3D bioprinted tissues, and *in vivo* mouse models to interrogate the precise mechanisms by which PIN1 controls fibroblast phenotypes and impact of these changes on tumor phenotypes and outcomes.

PO-114 STAT3 in cancer-associated fibroblasts promotes an immunosuppressive tumor microenvironment. Julia E. Lefler, Michael Ostrowski, Catherine MarElia-Bennett. Medical University of South Carolina, Charleston, SC.

One of the defining characteristics of pancreatic ductal adenocarcinoma (PDAC) is the formation of a dense stroma comprised of cancer associated fibroblasts (CAFs) and immune cell populations. This stroma is immunosuppressive and can act as a physical barrier against common therapeutic treatments. Attempts to therapeutically target the PDAC stroma have yielded contradictory results, suggesting both tumor promoting and tumor limiting roles for CAFs. These studies emphasize the need to understand important transsignaling pathways between CAFs, tumor cells, and the immune microenvironment. IL-6 is a pleiotropic cytokine involved in several physiological functions and its increased expression is strongly associated with poor

survival rates in PDAC patients. STAT3 is a major downstream target of IL-6, and its aberrant activation has been implicated in PDAC tumor progression and immune evasion. IL-6 expression and the IL-6/STAT3 signaling axis in PDAC has been characterized in epithelial tumor cells, however its stromal-specific function on PDAC has yet to be elucidated. We hypothesized that the STAT3 signaling axis in pancreatic CAFs contributes to the immunosuppressive and fibrotic phenotype seen with disease progression. Employing CreLoxP technology, the fibroblast specific protein-1 (*Fsp-Cre*) transgene was used to conditionally delete STAT3 in fibroblasts in the *PdxFlp; Kras$^{G12D;}$ p53 $^{frt/frt}$* (KPF) PDAC mouse model developed by our lab. Deletion of STAT3 in fibroblasts significantly increased the survival in a cohort of KPF mice compared to those with intact STAT3. In preliminary investigations, we found an increase in CD8+ T cell infiltration but a decrease in regulatory T cells in the STAT3-deleted cohort. We also observed a decrease in immunosuppressive M2 macrophage populations and an increase in M1 macrophages in the STAT3-deleted cohort. These preliminary results demonstrate a previously unexplored role of IL-6/STAT3 signaling in fibroblasts during PDAC progression.

PO-115 Effects of mesothelin exert on tumor microenvironment in pancreatic ductal adenocarcinoma. Dongliang Liu[1], Ethan Poteet, Zhengdong Liang, Emily Laplante, Lisa Brubaker, Sadhna Dhingra, Aleksandar Milosavljevic, Changyi Chen, Qizhi Cathy Yao. Baylor College of Medicine, Houston, TX.

Background: High mesothelin (MSLN) expression has been associated with poor prognosis in patients with pancreatic ductal adenocarcinoma (PDAC); however, the effect of MSLN exert on PDAC tumor microenvironment (TME) is largely unknown. **Methods:** MSLN over-expressed (MSLN-OE) and knocked-down (MSLN-KD) PDAC cells were established to investigate the MSLN-PD-L1 relationship in PDAC. Using an MSLN overexpressed Panc02 (Panc02-MSLN) cell line, we established an orthotopic PDAC mouse model. All mice were euthanized on day 27 after cell inoculation and the tumor-infiltrating leukocytes (TILs) were isolated for mass cytometry (CyTOF) study. The gene expression correlation between MSLN and a panel of immune cell markers was assessed by using a dataset that includes149 PDAC patients in the TCGA-PAAD cohort. **Results:** MSLN upregulated PD-L1 expression at both mRNA and protein levels in PDAC. MSLN was able to enhance PD-L1 transcription by recruitment of NF-kB P65 to the PD-L1 promoter. The Orthotopic implanted Panc02-MSLN group showed significantly increased tumor size, tumor weight, upregulated PD-L1, and reduced survival when compared with the vector control group. CyTOF analysis of the total $CD45^+$ hematopoietic cells infiltrated to the TME revealed 13 distinct cell subsets. In MSLN-high tumors, there is a significant expansion of neutrophils, eosinophils, PD-1^+CD8^+, and $CD8^+$NK cells. In addition, pan T cells, regulatory T cells (Treg), $CD8^+$, and PD-1^+CD4^+ cells were increased. A considerable decrease of tumor-associated macrophages (TAM), dendritic cells, B cells, NK cells, and a trend towards decreased $Ly6C^+CD8^+$ cells was also found. Within the TAM subset, a decreased $iNOS^+$M1 to Relm-a^+M2 ratio was detected. Furthermore, although there were increased $CD8^+$NK cells, we found more immunosuppressive markers such as PD-1, CTLA4, LAG3, KLRG1, and TIM3, and a decreased activation marker CD69 and transcription factor T-bet. Intriguingly, gene expression correlation analysis with 149 PDAC patients cohort in TCGA dataset showed that MSLN expression negatively correlates with the mRNA levels of the B220 marker of B cells, the F4/80 marker of macrophages, as well as the CD4, CD8, CD69, granzyme B, IL-2 and IFN-γ markers of T-cell activation. **Conclusions:** We showed here that

MSLN could significantly modulate the TME, possibly through the upregulation of PD-L1 expression. Compared with MSLN-low PDAC tumors, MSLN-high tumors recruit diverse immunosuppressive rather than immunoreactive leukocytes into tumor tissues to constitute an immunologically cold TME. Since targeting MSLN or PD-L1 as a monotherapy exhibited only modest objective response rates in the clinic, our findings may provide an alternative strategy by combining the depletion of MSLN with immune checkpoint inhibitors, which could result in improved treatment efficacy in PDAC.

PO-116 Deletion of *Arginase 1* in myeloid cells alters the pancreatic cancer microenvironment. Rosa E. Menjivar[1], Zeribe Nwosu[1], Wenting Du[1], Katelyn Donahue[1], Carlos Espinoza[1], Ashley Velez-Delgado[1], Kristee Brown[1], Wei Yan[1], Christopher Halbrook[2], Yaqing Zhang[1], Costas Lyssiotis[1], Marina Pasca di Magliano[1]. [1]University of Michigan, Ann Arbor, MI, [2]University of California Irvine, Irvine, CA.

Pancreatic ductal adenocarcinoma (PDA) is a deadly disease with a 5-year survival of only 10%. PDA is characterized by an abundant fibroinflammatory stroma that includes abundant fibroblasts and immune cells, mainly myeloid cells. Infiltrating myeloid cells express high levels of Arginase 1 (Arg1), an enzyme that metabolizes L-arginine. Conversely, $CD8^+$ T cells are scarce in PDA, and when present have an overwhelmingly exhausted phenotype. Whether myeloid cell Arginase is a key driver of immune suppression in pancreatic cancer is unknown. Here, we tested the hypothesis that myeloid cells in the tumor microenvironment mediate immune suppression in PDA through expression of Arg1. To test this hypothesis, we used a combination of genetically engineered pancreatic cancer mouse models and pharmacological approaches. Using a FlpO- and Cre-based dual recombinase system, we have generated a mouse model that develops pancreatic cancer spontaneously because of oncogenic Kras expression in the epithelium, while at the same time lacking Arg1 expression in the myeloid cell compartment ($Ptf1a\text{-}^{FlpO/+}$;$Kras^{Frt\text{-}STOP\text{-}Frt\text{-}G12D/+}$;LysMCre;$Arg1^{f/f}$). To complement the genetic model and inhibit the function of Arginase systemically, we used an Arginase inhibitor (CB-1158, *Incyte, Inc*.) alone and in combination with an immune checkpoint blockade (anti-PD1). Using these multiple approaches, we observed decrease progression to invasive disease in the genetic model, and sensitization to immune checkpoint treatment in the transplantation model. In both settings, changes in tumor growth were accompanied by an increase in CD8+ T cell infiltration and activation. These changes support the notion that myeloid Arg1 is mediator of immune suppression in PDA, and a potential therapeutic target.

PO-117 The role of Hippo signaling in stromal-epithelial interactions in acinar-to-ductal metaplasia and pancreatic cancer initiation. Julia Messina-Pacheco[1], Yasser Riazalhosseini[2], Zu-hua Gao[1], Alex Gregorieff[1]. [1]Department of Pathology, McGill University and the Research Institute of McGill University Health Centre, Montreal, QC, Canada, [2]Department of Human Genetics, McGill University and the McGill University and Genome Quebec Innovation Centre, Montreal, QC, Canada.

Background: Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer deaths with a 5-year survival rate of approximately 7%. PDAC may originate from acinar cell trans-differentiation into ductal-like cells, termed acinar-to-ductal metaplasia (ADM), triggered by chronic pancreatitis and/or mutations in K-Ras. The progression to PDAC is associated with a

dense fibrotic stroma, including cancer-associated fibroblasts (CAFs). YAP is a tension-stimulated CAF activator that promotes ECM stiffening, creating a permissive microenvironment for cancer progression. We hypothesize that the Hippo pathway may coordinate fibroinflammatory signals emanating from the stromal compartment during regenerative responses to acinar cell injury and progression towards PDAC. **Methods:** To resolve the transcriptional changes occurring during the transition to ADM and PDAC, we mapped the *in situ* expression of over 1800 RNA targets in patient-derived tissues using NanoString Technologies' Digital Spatial Profiling (DSP) technology. We also performed immune-profiling and evaluated Yap expression in human ADM by immunohistochemistry. To study the *in vivo* role of Hippo signaling in stromal cells, we conditionally deleted *Yap/Taz* in Collagen1a2-producing cells in a murine model of caerulein-induced pancreatitis, which recapitulates many of the features associated with human ADM. I will analyze the resulting phenotype by immunostaining for metaplastic, proliferative, immune and stromal markers. **Results**: DSP analysis revealed genes implicated in fibroblast activation, epithelial-to-mesenchymal transition (EMT), neutrophil activation and IFNɣ signaling as potential key drivers of ADM. I will further evaluate the expression of candidate genes and survey *Yap* expression at the single cell level in human ADM tissue by multiplexed RNAscope *in situ* hybridization. We found up-regulation of CD4+ and CD8+ T cells in ADM, and an increasing trend of neutrophil and macrophage accumulation in the progression from normal parenchyma to ADM to PDAC. **Conclusions:** This work will provide an in-depth understanding of epithelial-stroma crosstalk in ADM and a foundation for the development of new therapeutic strategies for treating non-invasive precursor lesions like ADM, thereby preventing pancreatic cancer progression. **Source of Funding:** This research is supported by the Fonds de Recherce du Quebec – Santé (FRQS), Canadian Institutes of Health Research (CIHR) and the Research Institute of the McGill University Health Centre (RI-MUHC).

PO-118 The tumor immune microenvironment is decisive in the survival of pancreatic ductal adenocarcinoma. Hosein M. Aziz, Lawlaw Saida, Willem de Koning, Andrew Stubbs, Yunlei Li, Casper H. J. van Eijck, Dana A. M. Mustafa. Erasmus University Medical Center, Rotterdam, Netherlands.

Background: Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy associated with a poor prognosis. Only 10% of the patients survive longer than five years. So far, factors underlining long-term survivorship in PDAC are not well understood. Therefore, we aimed to identify the key players in the tumor immune microenvironment (TIME) associated with long-term survivorship in PDAC patients. **Methods:** The immune-related gene expression profiles of surgically resected PDAC tumors of patients who survived and remained recurrence-free of disease for > 3 years (long-term survivors, n=10) were compared to PDAC tumors of patients who had survived ≤ 6 months (short-term survivors, n=10) due to tumor recurrence. Samples were profiled using the nCounter® PanCancer Immune Profiling Panel of NanoString Technology. Validation was performed by spatial analysis of immune cells using the GeoMx™ Digital Spatial Profiler. **Results:** Tumor-infiltrating B cells were found to be significantly increased in the TIME of long-term survivors by gene expression profiling (p=0.018). The high tumor infiltration of B cells was confirmed by spatial protein profiling (p=0.049). This increase was accompanied by more T cells and antigen-presenting cell infiltration. Moreover, the activated immune cells were found to infiltrate in between tumor cells as well as in stromal areas

in long-term survivors. In contrast, the TIME of short-term survivors was characterized by a high density of immunosuppressive cells like CD25 and regulatory T cells infiltrating in a highly fibrotic vicinity. **Conclusion:** This is the first comprehensive study that connects the immune landscape of gene expression profiles and protein spatial infiltration with the survivorship of PDAC patients. We found higher infiltration of B cells in TIME of long-term survivors which highlights the importance of targeting B cells and B cell-based therapy for future personalized immunotherapy in PDAC patients.

PO-119 DFMO mediated improvement in survival of an orthotopic model of pancreatic cancer is associated with modulating immune suppression in the tumor microenvironment.
Sai Preethi Nakkina[1], Sarah B. Gitto[2], Veethika Pandey[2], Jordan M. Beardsley[1], Michael W. Rohr[1], Jignesh G. Parikh[3], Otto Phanstiel[4], Deborah A. Altomare[1]. [1]University of Central Florida, Orlando, FL, [2]University of Pennsylvania, Philadelphia, PA, [3]Orlando VA Medical Center, Orlando, FL, [4]College of Medicine, University of Central Florida, Orlando, FL.

There remains an urgent need to target pancreatic tumor cells using innovative strategies. KRAS and MYC, are important oncogenes in pancreatic ductal adenocarcinoma (PDAC) which pose a challenge to successful treatment of PDAC. Our previous studies have shown that inhibition of ornithine decarboxylase 1 (ODC1) using difluoromethylornithine (DFMO) decreases MYC expression and tumorigenesis. GW5074 can modulate RAF1, a downstream effector of KRAS. Here we test the responsiveness of pancreatic tumor cells treated with DFMO alone and in combination with GW5074. We used an orthotopic animal model of pancreatic tumor using KRas-driven murine pancreatic cancer cells (PanO2) to test the effects of these compounds on tumor microenvironment and overall survival. Cellular and molecular changes in the tumor microenvironment were assessed using immunohistochemistry. The results showed an inhibition of pancreatic cancer cell viability in DFMO and DFMO+GW5074 treatment groups *in vitro*, with a significant decrease in tumor weight compared to control treatment group *in vivo*. However, in terms of overall survival, DFMO alone resulted in a dramatic increase in survival compared to control treatment group. Interestingly, GW5074 alone or DFMO in combination with GW5074 did not result in a detectable effect on survival. Further investigation of immune cells in the tumor microenvironment revealed that standalone DFMO treatment was associated with an increase in infiltration of macrophages, T cell costimulatory marker CD86 and T cell markers (CD3, CD4 and CD8) compared to control and GW5074 treated groups. Additionally, DFMO is associated with decreased MYC expression compared to control and GW5074 treated groups. In conclusion, DFMO decreased MYC expression and associated immune suppression in the PDAC microenvironment. In contrast standalone GW5074 treatment resulted in maintenance of MYC expression and worse survival. In conclusion, the present study highlights the success of DFMO in PDAC treatment in part through downregulation of MYC and a decrease in associated immune suppression.

PO-120 Differential expression of polyamine pathways in human pancreatic tumor progression and effects of polyamine blockade therapy on the *in vivo* pancreatic tumor microenvironment.
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Pancreatic cancer is the fourth leading cause of cancer death in the United States, with a five-year survival rate of less than 8%. Existing therapies have failed to improve pancreatic ductal adenocarcinoma (PDAC) patient prognosis. The dense desmoplastic reaction which occurs in PDAC makes it challenging for drugs and immune cells to infiltrate the fibrotic barrier. There is a need to exploit lesser explored targets in PDAC that can influence both the tumor and its microenvironment. One such avenue could be via targeting polyamine metabolism which is upregulated in pancreatic tumors. Though aberrant polyamine upregulation in pancreatic tumors has been known for decades, there has been little progress in translating this information into a PDAC therapeutic strategy. Additionally, there is a dearth of information regarding the dysregulation of polyamine metabolism in human PDAC and its association with clinical outcomes. Thus far, preclinical studies targeting polyamines using polyamine blockade therapy (PBT) has improved survival of pancreatic tumor bearing mice. Literature shows effectiveness of PBT in eliciting an anti-tumor immune response in other tumor types. Whether these results translate to the immune-privileged PDAC microenvironment need to be determined. The present study explores polyamine gene expression in human PDAC samples by mRNA expression analysis of frozen PDAC and Pancreatic intraepithelial neoplasia (PanIN). The Cancer Genome Atlas in the public domain was used to identify clinical outcomes of PDAC patients associated with select polyamine gene expression. Further, the anti-tumor effects of PBT and associated tumor microenvironment changes were identified using in vivo PDAC models and histological assessment. Polyamine dysregulation was found to be evident in human PDAC progression. Also, increased expression of certain polyamine-related genes was associated with poorer survival of pancreatic cancer patients. When targeting polyamines using PBT in immunocompetent C57Bl/6 mice with Pan02 tumor cells injected in the pancreas, PBT significantly increased overall survival. PBT also resulted in an increase in the infiltration of macrophages (F4/80) and expression of T-cell co-stimulatory marker (CD86) as assessed by immunohistochemistry and further quantification of imaging. Based on these changes, we hypothesized that PBT could prime the tumor microenvironment to be more susceptible to existing therapeutics. In conclusion, targeting polyamines using PBT results in increased survival and immune modulation in PDAC.

PO-121 Investigating the role of human cancer-associated fibroblasts in pancreatic cancer invasion using patient-derived PDAC organoids. Bernat Navarro-Serer, Kenna Sherman, Laura D. Wood. Johns Hopkins University School of Medicine, Baltimore, MD.

Organoid cultures have emerged as a promising research model as they more accurately recapitulate *in vivo* tumor features and provide a system to study cancer invasion, among others. We have previously reported the development of a human pancreatic cancer organoid model using surgically resected PDAC tumors. This model has allowed us to characterize molecular alterations critical for invasion. Culturing PDAC organoids in collagen I gels identified the ability of organoids to invade using two distinct, morphologically defined invasive phenotypes. Interestingly, invasion of PDAC organoids in collagen I decreased after culturing and passaging them in Matrigel, suggesting the microenvironment plays a crucial role in promoting invasion. To identify factors that increase PDAC organoid invasion, we sought to

investigate the relationship between human PDAC organoids and patient-derived cancer-associated fibroblasts (CAFs). CAFs are a key component of the PDAC microenvironment, characterized by their ability to perform a variety of functions, including deposition and remodeling of ECM as well as promoting tumor growth, among others. CAFs may induce tumor-suppressive or tumor-promoting effects, and the role of CAFs subtypes in PDAC invasion has not been studied extensively. CAFs are also a heterogeneous group of cells: two mutually exclusive and reversible subtypes of CAFs have been discovered (inflammatory and myofibroblastic, also known as iCAFs and myCAFs respectively) driven on paracrine and juxtacrine signaling mechanisms. We have investigated the impact of patient-derived CAFs on PDAC organoid invasion by using conditioned media of CAFs in monolayers, which expand as myCAFs. After 2-3 days of culture, media was collected and human PDAC organoids (100-150 total) from 5 patients were allowed to grow and invade in collagen I gels with or without the addition of CAF conditioned media. Our results show conditioned media of CAFs increases the invasiveness of PDAC organoids but does not increase the percentage of organoids invading. In order to identify potential heterogeneity between primary CAF cultures from different patients, we performed time-lapse analyses of organoids in collagen I gels with conditioned media from 3-4 patient-derived CAFs. Interestingly, we did not find any significant differences between primary CAF cultures in inducing PDAC organoid invasion, suggesting that the ability of CAFs to induce tumor cell invasion does not vary widely between different patients. However, we did observe some patient-derived organoids did not increase invasiveness upon addition of conditioned media from any of the CAF cultures, showing heterogeneity in tumor cells from different patients in response to invasion-inducing factors secreted by CAFs. Future studies will examine the ability of iCAFs to induce invasion in our organoid model and the differences between the secretomes of myCAFs and iCAFs.

PO-122 Combined CDK and BET inhibition reprograms the tumor and stromal compartments to enhance anti-tumor immunity in immunologically-cold CDKN2A-deficient pancreatic cancer. Brian M. Olson, Alison J. Thomas, Michael B. Ware, Gregory B. Lesinski. Emory University, Atlanta, GA.

Background: Pancreatic ductal adenocarcinoma (PDAC) has a highly immunosuppressive tumor microenvironment (TME). The immune landscape of these tumors is further influenced by cross-talk with fibroblasts present in stroma. Importantly, genomic features also influence immune and stromal composition within PDAC and may provide opportunity for personalized immunotherapy. Given the central role of CKDN2A in PDAC (being lost in up to half of patients, and the second most commonly alteration across all metastatic disease), we hypothesize that the CDKN2A-deficient PDAC TME can be adapted for therapeutic targeting to sensitize PDAC to immunotherapy. **Methods:** Bioinformatic analysis of PDAC samples from the TCGA database evaluated how CDKN2A loss alters expression of T cell-inflamed gene signatures. Preclinical *in vitro* and *in vivo* studies were conducted with CDKN2A-targeted CRISPR-Cas9-edited luciferase-expressing KPC cells (KPC-luc) to generate isogenic KPC-luc$^{\Delta CKDN2A}$ cells. The BET inhibitor JQ-1 and CDK inhibitor palbociclib were used to evaluate impact on apoptosis and immune-related signaling using isogenic KPC-luc cells and patient-derived PDAC organoids. Spheroids were used to evaluate impact of CDK/BET inhibition on cross-talk with pancreatic cancer-associated fibroblast (CAF) spheroids. Finally, *in vivo* studies tested the ability of CDK/BET inhibition to enhance T cell migration, and delay tumor growth when combined

with PD-L1 blockade. **Results:** CDKN2A loss was enriched in tumors lacking a T cell-inflamed gene signature. Similarly, CDKN2A loss decreased T cell migration *in vitro* and into the KPC-luc$^{\Delta CDKN2A}$ TME *in vivo*. Loss of CDKN2A rendered KPC-luc cells susceptible to direct apoptosis from dual CDK/BET inhibition. Combined CDK/BET inhibition at sublethal doses also modulated immune phenotype of tumors, inducing DNA damage and cGAS/STING to increase type I interferon and T cell migration *in vitro*. Additionally, treatment with BETi alone or combined with CDKi also modulated checkpoint ligands on KPC-luc cells and patient-derived organoids. In stroma, treatment of PDAC CAFs with JQ-1 polarized CAFs towards a myofibroblast CAF (myCAF) phenotype. This could also be mediated via cross-talk between tumor and CAF cells, as JQ-1-treated KPC-luc$^{\Delta CDKN2A}$ cells or supernatants cultured with CAF spheroids also induced myCAFs. This cross-talk was bidirectional, as supernatants from BETi-treated CAFs modulated expression of checkpoint ligands on KPC-luc cells. *In vivo*, combined BET/CDK inhibition increased T cell infiltration into PDAC tumors, and when combined with PD-L1 blockade diminished tumor growth. **Conclusion:** While CDKN2A loss promotes an immunosuppressive PDAC TME, it also renders tumors susceptible to BET/CDK inhibition. This approach polarizes CAF cells towards a myCAF phenotype, directing cross-talk between tumors and stroma to promote a more immunogenic phenotype. This suggests combined CDK/BET inhibition may be a personalized therapeutic approach for patients with CKDN2A-deficient PDAC, and a means to sensitize PDAC to immunotherapy.

PO-123 Development of a 3D biomimetic metastatic liver niche model for pancreatic cancer. Mahsa Pahlavan[1], Weikun Xiao[2], Flora Eun[2], Chang-Il Hwang[3], Reginald Hill[2]. [1]Department of Biomedical Engineering, University of Southern California, Los Angeles, CA, [2]Lawrence J. Ellison Institute for Transformative Medicine of USC, University of Southern California, Los Angeles, CA, [3]Department of Microbiology and Molecular Genetics, College of Biological Sciences, University of California Davis, Davis, CA.

Pancreatic ductal adenocarcinoma (PDAC) is the 3rd leading cause of cancer death in the United States with a 5-year survival rate of only 7%. Early diagnosis is very difficult and thus 53% of PDAC patients are diagnosed after metastasis has already occurred with liver being the most frequently affected site. Therefore, it is urgent to understand the mechanism that leads to PDAC metastasis in liver in order to develop potential therapeutics. Research shows that environment that comprises the metastatic niche has unique features that facilitate tumor growth and chemoresistance. Differences between the tumor microenvironment (TME) of the primary site and the metastatic site may be a key reason why metastatic tumors are highly resistant to standard treatment. However, most current models only recreate the primary tumor environment, while a proper model which recapitulates the key features of the metastatic niche to study liver metastasis (LM) is still missing. To address this problem, we aim to design a biomimetic model that specifically recapitulates the liver premetastatic niche (PMN). Our 3D model accomplishes this by using 1) a collagen rich extracellular matrix (ECM) that mimics the metastatic site, 2) LM-derived fibroblasts, 3) and LM organoids derived from a mouse model of PDAC. Our preliminary results showed that primary PDAC organoids require CAFs derived from primary PDACs to exhibit chemoresistance. In contrast, LM organoids did not require primary CAFs to exhibit the same level of chemoresistance. This suggests that there are cell intrinsic factors that promote chemoresistance in LM organoid in addition to possible cell extrinsic factors from the PMN. In this research we investigate the role of LM-derived CAFs and PDAC-derived exosomes

in liver PMN development and study their effect on growth and chemoresistance of LM organoids. Results of this research will help us to develop strategies specifically tailored to overcome the factors which promote chemoresistance in the PMN.

PO-124 EZH2 blockade overcomes suppression of the proinflammatory senescence-associated secretory phenotype in the pancreas and drives NK cell-mediated pancreatic tumor responses. Loretah Chibaya[1], Yvette Lopez-Diaz[1], Haibo Liu[1], Katherine C. Murphy[1], John P. Morris IV[2], Yu-jui Ho[2], Janelle Simon[2], Wei Luan[2], Amanda Kulick[2], Lakhena Leang[1], Elisa de Stanchina[2], Lihua J. Zhu[1], Scott W. Lowe[2], Marcus Ruscetti[1]. [1]University of Massachusetts Medical School, Worcester, MA, [2]Memorial Sloan Kettering Cancer Center, New York, NY.

T cell-targeting immunotherapies that produce durable and sometimes curative responses in other malignancies have failed in pancreatic ductal adenocarcinoma (PDAC) due to poor T cell infiltration and tumor immunogenicity. We and others have demonstrated that cellular senescence and its associated secretory phenotype (SASP), which leads to production of proinflammatory cytokines, chemokines, and growth factors, can be a powerful way to reactivate a different type of Natural Killer (NK) cell immunity. Recently, we found that RAS pathway targeting MEK and CDK4/6 inhibitors can induce senescence in KRAS mutant lung tumor and PDAC models; however, therapy-induced senescence (TIS) led to NK cell-mediated tumor regressions only in the lungs. Here we set out to understand how the pancreas tumor microenvironment (TME) suppresses NK immunity and develop strategies to harness NK cell surveillance for PDAC immunotherapy. Syngeneic murine KRAS-driven lung tumor and PDAC cells were transplanted into lungs, pancreas, or liver of C57BL/6 mice. Following 2-week treatment with the MEK inhibitor trametinib and CDK4/6 inhibitor palbociclib (T/P) to induce senescence, immune responses were assessed by flow cytometry, and the SASP profile assessed by RNA- and ATAC-seq analysis. An NK1.1-targeted antibody was also used to deplete NK cells and evaluate treatment efficacy. shRNA-mediated EZH2 knockdown and treatment with EZH2 methyltransferase inhibitors in murine and human PDAC cells and mouse models was used to determine the role of EZH2 in SASP regulation by qPCR and cytokine array. The effects of EZH2 blockade on NK cell immunity were determined in co-culture migration and cytotoxicity assays *in vitro* and in PDAC transplant models *in vivo* by flow cytometry. The efficacy of T/P treatment in combination with EZH2-targeting shRNAs or inhibitors was measured by ultrasound tumor measurements and survival in PDAC-bearing animals. Tumors propagated in the pancreas TME display transcriptional repression and reduced chromatin accessibility of SASP transcriptional activators (NF-KB, IRFs) and factors necessary for NK cell activity (IL-15,-18) and chemotaxis (CCL2,-7,-8; CXCL9,-10) following TIS, and do not undergo anti-tumor NK immune surveillance as compared to tumors grown in the lungs and liver. We identified induction of EZH2 and repression of its targets, including key SASP factors and regulators, specifically in tumor cells grown in the pancreas TME. Genetic or pharmacological inhibition of EZH2 enhanced proinflammatory SASP signaling and resulted in NK cell infiltration and anti-tumor cytotoxicity in PDAC models. Remarkably, EZH2 knockdown in combination with T/P treatment led to complete tumor regressions in PDAC-bearing mice that were reversed following NK cell depletion. These results demonstrate that EZH2 mediates transcriptional repression of the proinflammatory SASP in the pancreas TME,

and that EZH2 blockade in combination with TIS could be a powerful means to reactivate absent NK cell surveillance in PDAC to achieve immune-mediated tumor control.

PO-125 The role of KDM6A in pancreatic cancer immune microenvironment. Lin Jin, Jing Yang, Zhujun Yi, Hong S. Kim, Feng Tian, Jiaqi Shi. University of Michigan, Ann Arbor, MI.

Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer death in the United States, with a 10% 5-year survival rate. Immunosuppressive myeloid cells, including tumor-associated neutrophils (TANs), contribute to tumor development and treatment resistance. Lysine (K)-specific demethylase 6A (KDM6A) is one of the most frequently mutated epigenetic genes and a tumor suppressor in PDAC. However, the molecular mechanisms by which KDM6A contributes to PDAC development and whether KDM6A impacts the tumor immune microenvironment are unknown. This study established a genetically engineered pancreas-specific *Kdm6a*-knockout *Ptf1a*^{Cre}*;LSL-Kras*$^{G12D/+}$*;LSL-p53*$^{R172H/+}$ (KPC) PDAC mouse model to investigate the influence of KDM6A loss on PDAC development and tumor immune microenvironment. We found that KDM6A loss accelerated PDAC progression and increased metastases. Pancreata of mice with *Kdm6a* deficiency developed aggressive undifferentiated PDACs. Additionally, KDM6A loss led to increased infiltration of TANs and neutrophil extracellular traps (NETs) formation. Mechanistically, we used Bru-seq technology to investigate the impact of KDM6A on nascent RNA transcription. We demonstrated that many chemotactic cytokines related to neutrophil recruitment, specifically CXCL1, were upregulated in KDM6A-knockout PDAC cells. We further confirmed increased CXCL1 mRNA and protein levels in KDM6A-deficient human and mouse PDAC cells. In addition, immunohistochemical staining also confirmed the upregulated CXCL1 expression in both human and murine PDAC cells with KDM6A loss. TANs were found to express CXCR2, the receptor of CXCL1, by immunofluorescent analysis. To further confirm that cancer cells with KDM6A loss can attract neutrophils *in vitro*, we performed chemotaxis assays using both human neutrophils derived from PLB-985 cells and mouse primary neutrophils isolated from bone marrow and conditioned media from KDM6A- knockout or knockdown PDAC cells. We found that PDAC cells with KDM6A loss promoted neutrophil recruitment *in vitro* compared to KDM6A-retained PDAC cells. Furthermore, the CXCL1 neutralizing antibody reversed the chemotactic property of KDM6A-deficient PDAC cells, confirming that CXCL1 is the primary chemokine mediating the neutrophil recruitment. In summary, these findings shed light on the mechanism by which KDM6A loss promotes PDAC development, regulates tumor immune microenvironment, and suggests that the CXCL1-CXCR2 axis may be a candidate target for the treatment of PDAC, especially those with KDM6A mutations.

PO-126 Loss of HIF1A decreases resistance to radiation and invasiveness in pancreatic ductal adenocarcinoma. Kevin J. Tu[1], Sanjit K. Roy[1], Binny Bhandary[2], Amit Sawant[1], Hem D. Shukla[1]. [1]University of Maryland School of Medicine, Baltimore, MD, [2]University of Maryland, Baltimore, Baltimore, MD.

Pancreatic ductal adenocarcinoma (PDAC) is the fourth most lethal cancer in the United States with an estimated 60,430 new cases and 48,220 deaths in 2021. PDACs are characterized by extensive desmoplastic stroma and severe hypovascularity, resulting in an intra-tumoral hypoxic

microenvironment. In response to hypoxic stress, cancer cells set off many adaptive responses including metabolism regulation, cell survival, and inflammation through the stabilization and activation of the hypoxia-inducible factor (HIF) family of transcription factors. In this study, we used the gene-editing tool CRISPR-cas9 to knock out HIF1A and investigate whether HIF1A affects the radioresistant and invasive characteristics of PDACs in a KPC cell line model. To first observe how HIF1A affects PDAC radioresistance, we performed a clonogenic survival assay with increasing doses of radiation on both wild-type and HIF1A knockout (KO) cells treated and untreated with $CoCl_2$-induced hypoxia conditions (100 uM and 200 uM $CoCl_2$). Our data showed that under hypoxia, KO cells exhibited significant cell death when treated with 6, 8, and 10 Gy of radiation as compared to wild-type KPC cells, emphasizing the role of HIF1A in radiation resistance. In addition, to understand the role of HIF1A in regulating the invasive behavior of PDACs, we performed a cell proliferation assay on wild-type and KO KPC cells. HIF1A KO cells treated with $CoCl_2$ exhibited significantly reduced proliferation compared to wild-type cells also treated with $CoCl_2$ ($p<0.01$), though no significant difference was observed between untreated HIF1A KO and untreated wild-type cells ($p>0.05$). Because western blot demonstrated increased HIF1A expression in wild-type cells following $CoCl_2$ treatment, our results provided evidence for the role of HIF1A activation in promoting PDAC invasiveness. Through western blot, we also confirmed the association between HIF1A expression with p53 degradation in PDAC. We used COREMINE, a literature mining tool, to map a direct interaction between HIF1A with KRAS in PDACs ($p=0.000016$). We propose HIF1A as a switch to activate KRAS and degrade p53 under hypoxic conditions in PDAC proliferation. Thus, modulating the HIF1A switch may be an important mechanism to reduce the tumor-promoting microenvironment and inhibit cancer growth.

PO-127 A uPA/uPAR axis in both the tumor cell and stromal compartment drives PDAC disease progression. Yi Yang, Sara R. Abrahams, Aditi Kothari, Harshi Matada, Keely Davey, Alisa S. Wolberg, Matthew J. Flick. University of North Carolina, Chapel Hill, Chapel Hill, NC.

Pancreatic ductal adenocarcinoma (PDAC) is a lethal solid tumor malignancy with a 5-year survival rate of 9%. In both patients and animal models of disease, PDAC is associated with robust coagulation system activity. Intriguingly, in addition to being a rich source of procoagulant factors, PDAC tumors highly express fibrinolytic system components. Supporting this concept, urokinase plasminogen activator (uPA) and uPA receptor (uPAR) expression positively correlates with reduced overall patient survival. Here, we tested the hypothesis that the expression and activity of plasminogen activation (PA) system components are functionally linked to PDAC tumor growth and disease progression. We generated C57Bl/6-derived KPC (*i.e.*, $KRas^{G12D}$, $TRP53^{R172H}$) PDAC cell lines in which uPA and uPAR were knocked out using CRISPR-Cas9. We then analyzed orthotopic tumor growth and experimental metastasis in mice carrying null or functional mutations in uPA, uPAR, or plasminogen to evaluate the interplay of PA components derived from tumor cells and/or stromal cells in mediating PDAC progression. Although both KPC cell CRISPR variants retained procoagulant function, elimination of tumor cell uPA or uPAR yielded significantly smaller tumors when compared to Cas9 control tumor cells in wildtype mice. Similarly, the growth of WT KPC tumor cells in C57Bl/6 background uPA-KO or uPAR-KO mice also resulted in reduced tumor growth. To our surprise, the metastasis potential of WT KPC tumor cells in uPA-KO or uPAR-KO mice did not change when

compared to wildtype mice. Regarding to the uPA/uPAR axis downstream effector plasminogen, the growth of WT KPC tumors in plasminogen-KO mice was also significantly reduced, but not to the same extent as when eliminating uPA or uPAR. In addition, eliminating plasminogen drastically reduced WT KPC tumor cells metastasis potential. In conclusion, our data suggest a mechanism whereby uPA functions through uPAR in both the tumor cell and stromal cell compartments to promote PDAC progression through plasminogen-dependent and -independent mechanisms.

PO-129 Targeting CCR1 reprograms tumor associated macrophages in pancreatic cancer. Yaqing Zhang, Kristee L. Brown, Wei Yan, Zeribe C. Nwosu, Eileen K. Carpenter, Katelyn L. Donahue, Ashley Velez-Delgado, Sion Yang, Marina Pasca di Magliano. University of Michigan, Ann Arbor, MI.

The tumor microenvironment of pancreatic ductal adenocarcinoma (PDA) includes abundant fibroblasts and infiltrating immune cells, the latter largely immunosuppressive. We previously showed that targeting regulatory T cell (Treg), a prevalent T cell population in pancreatic cancer, failed to relieve immunosuppression and led to accelerated tumor progression. We discovered that Treg depletion reprogrammed tumor associated fibroblasts and increased immunosuppressive myeloid cell recruitment, an effect that was partially mediated by CCLs/CCR1signaling. Thus, we sought to investigate the potential therapeutic effect of targeting CCR1 in pancreatic cancer. By single cell RNA sequencing, we found CCR1 to be mainly expressed by tumor associated macrophages (TAMs) and neutrophils (or granulocytes) in both human and mouse PDA. We then orthotopically transplanted syngeneic mouse pancreatic cancer cells in CCR1 knockout hosts, and observed reduced tumor growth which was rescued by CD8 T cell depletion. Histological analysis showed elevated Granzyme B expression in infiltrating T cells, as well as an increase in cleaved caspase 3 positive cancer cells in tumors implanted in $Ccr1^{-/-}$ mice. Through cytometry by time of flight (CyTOF) and co-immunofluorescence we also discovered that TAMs in tumors implanted in $Ccr1^{-/-}$ mice expressed less Arginase 1 and CD206 -both markers of immunosuppressive macrophages- compared to TAMs in wild type tumors. Thus, our data is consistent with the notion that tumor associated macrophages lacking CCR1 expression are less immunosuppressive, consequently allowing increased CD8 T cell-mediated anti-tumor immunity. We are currently exploring combination approaches targeting CCR1 in pancreatic cancer.

PO-130 Macropinocytosis at the nexus of crosstalk in the pancreatic tumor microenvironment. Yijuan Zhang[1], M. Victoria Recouvreux[1], Michael Jung[1], Koen Galencamp[1], Yunbo Li[2], Olga Zagnitko[1], David Scott[1], Andrew Lowy[2], Cosimo Commisso[1]. [1]Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, [2]University of California San Diego, La Jolla, CA.

A feature of tumor cells is the acquisition of nutrients via macropinocytosis, an endocytic form of protein scavenging that functions to support metabolism and proliferation in the context of a nutrient-depleted tumor microenvironment. Here, we provide evidence that macropinocytosis is also operational in the tumor stroma. We find that glutamine deficiency triggers macropinocytic uptake in patient-derived cancer-associated fibroblasts (CAFs) from pancreatic ductal adenocarcinoma (PDAC) tumors. Mechanistically, stromal macropinocytosis is dependent on a

CaMKK2-AMPK signaling axis that activates Rac1. We find that these signaling events are potentiated via an enhancement of cytosolic Ca^{2+} caused by glutamine limitation. Interestingly, glucose deprivation, which robustly enhances AMPK activation, does not activate Rac1 and fails to induce uptake in CAFs. We determine that the selectivity of the observed macropinocytic response is mediated by the glutamine depletion-induced upregulation of ARHGEF2, a Rho/Rac guanine nucleotide exchange factor. We elucidate that macropinocytosis has dual function in CAFs – it serves as a source of intracellular amino acids that sustain CAF viability, and it provides extracellular amino acids that promote tumor cell survival. By specifically ablating macropinocytosis in the stroma, we demonstrate that macropinocytosis-deficient CAFs are unable to enhance PDAC tumor growth. Current efforts are focused on how different metabolic inputs in the tumor microenviroment can differentially regulate the CaMKK2-AMPK signaling axis in stromal cells. These results highlight the functional role of macropinocytosis in the tumor stroma and provide a mechanistic understanding of how nutrient deficiency can control stromal protein scavenging.

PO-131 The role of liver endothelium on pancreatic cancer growth. Wei Zhang[1], Michel'le Wright[1], Moeez Rathore[1], Ali Vaziri-Gohar[1], Jordan Winter[2], Rui Wang[2]. [1]Case Western Reserve University, Cleveland, OH, [2]Case Western Reserve University/University Hospitals Cleveland Medical Center, Cleveland, OH.

Introduction: Pancreatic ductal adenocarcinoma (PDAC) has the highest mortality rate among major cancers in the United States, and the 5-year survival rate for patients with metastatic PDAC (mPDAC) is only at 3%. Past studies have shown that the paracrine secretion of soluble factors by endothelial cells (ECs) created a unique niche and promoted the survival of cancer cells (cell growth or chemoresistance) in other types of cancer. The liver is the main site of distant metastases in mPDAC, but the influence of the liver EC microenvironment on mPDAC has not been elucidated. In this study, we determined the paracrine effects of liver ECs on the survival of PDAC and identify involved mechanism. **Methods:** Primary liver ECs were isolated from non-neoplastic liver tissues to mimic the liver EC microenvironment. Conditioned medium (CM) from liver ECs were collected and then applied to PDAC cells, with CM from PDAC as control CM. Effects of CM on PDAC cell proliferation were measured by the MTT assay. Changes in phosphorylation of receptor tyrosine kinases (RTK) between PDAC CM and EC CM treated PADC cells were determined by a Phospho-RTK Array kit and then validated by Western blotting. Involved RKTs were blocked by antibodies for determining their roles in mediating EC effects on PDAC cells. Lastly, A xenograft tumor model was used to establish PDAC tumors and then treated xenograft mice were subcutaneously injected by either PADC CM or EC CM, and tumors growth was monitored and recorded to evaluate the effect of ECs on PADCs. **Results:** Compared to PDAC CM, EC CM promoted proliferation in 4 different PDAC cells. We found that human epidermal growth factor receptor 3 (HER3 or ERBB3) was only expressed and activated in BxPC-3 cells (HER3+ve), in which the HER3-AKT signaling pathway was activated by EC CM. Furthermore, blocking HER3 activation with a humanized HER3 antibody, seribantumab, completely blocked EC CM-induced AKT activation and cell proliferation. Moreover, depletion of neuregulin (NRG) from EC CM attenuated HER3-AKT activation and indicated that EC-secreted NRG might play a role in promoting PDAC growth. It is interesting that ERK activation was also observed but was not affected by HER3 inhibition. It implied that EGFR signaling pathway might also be involved in EC CM induced PDAC cell growth.

Moreover, the combination of cetuximab, trastuzumab and seribantumab yielded the best inhibitory ability on EC CM promoted cell growth as compared to antibody alone or the combination of two of them. Finally, EC CM promoted PDAC tumor growth was also observed in BxPC-3 derived xenograft mouse model. **Conclusions:** Our results demonstrated that liver EC-secreted factors promoted PDAC growth either *in vitro* or *in vivo*, and HER3 was expressed in a subset of PDAC cells and mediated EC-induced proliferation. Moreover, EGFR pathway may also play a role in EC induced cell growth and needs to be addressed in the future study. Our findings suggest a potential of using the combination of HER antibodies/inhibitors for treating patients with HER3+ve mPDACs.