Aiman **Abzhanova** **PITM** **Curriculum in Toxicology and Environmental Medicine (CiTEM)**

Novel whole wood smoke exposure and imaging system for human bronchial epithelial cells cultured at the air-liquid interface.

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Wildland fire emissions are increasingly important contributors to air pollution. Wood smoke contributes 40% of primary emissions of fine particulate matter in the US, and its contribution is projected to increase. Epidemiological studies support the link between acute exposure to wood smoke and cardiopulmonary morbidity and mortality. Wood smoke is a complex mixture of gaseous, volatile, and particulate combustion products. The conventional approach to studying the toxicity of wood smoke in vitro relies on exposure of submerged cell cultures to smoke condensates and particle extracts, which does not fully recapitulate the complexity of wood smoke exposure, especially to its volatile fraction. A more relevant model to study the adverse health effects of the inhaled air pollutants uses fully-differentiated primary human bronchial epithelial cells cultured at the air-liquid interface (HBEC-ALI), which more accurately represents the in vivo morphology of bronchial epithelium. Disruption of redox homeostasis is often cited as an initiation mechanism for air pollution-induced adverse outcomes. We utilize a redox-sensitive sensor coupled with high-temporal resolution microscopy, to monitor the redox events in HBEC-ALI in response to wood smoke. Wood smoke is generated by the combustion of Red Oak in a quartz tube furnace. Generated emissions are conditioned with temperature, humidity, and carbon dioxide and delivered to a custom-built exposure chamber that allows confocal imaging of HBEC-ALI. Early results demonstrate oxidation of the glutathione pool, a marker of oxidative stress, in response to freshly generated wood smoke indicating the feasibility of studying redox events in HBEC-ALI during exposure to wood combustion emissions.

Whitney **Bell** **CCBTP** **Cell Biology & Physiology**

Revealing the role of Gastrokine2 in pancreatic cancer development

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Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers with a 5-year survival rate of only 10% and is one of the top five leading causes of cancer-related deaths for men and women in the United States. These dire statistics underscore the need for a better understanding of the mechanisms that promote pancreatic cancer initiation and progression. Recent scRNA seq work in murine models has identified Gastrokine2 (Gkn2) as a gene of interest in pancreatic cancer. GKN2 is a secreted gastric protein that is de novo expressed in the human and mouse pancreas during the development of pancreatic disease. Gastrokine2 has been shown to play tumor suppressive roles in gastric cancer though its role in the initiation and progression of PDAC remains unknown. I hypothesize that GKN2 has a functional role in the development of PDAC as knockdown of Gkn2 in our preinvasive and PDAC murine cell lines reveal multiple significantly affected pathways that have prognostic implications. Specifically, Gkn2 knockdown affects gastric factors already implicated in PDAC, endodermal lineage genes that comprise the classical PDAC subtype signature, as well as axonal guidance factors. With inducible shRNA systems to control Gkn2 expression in our in vitro and in vivo models, we propose to investigate how GKN2 affects pancreatic cancer development and progression.

Emma **Bouck** **PITM** **Pathobiology and Translational Science**

Oral contraceptives do not alter endothelial cell procoagulant activity

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Background: Women taking combined oral contraceptives (OCs) have a 2-5-fold increased risk of venous thromboembolism (VTE). Previous studies have observed procoagulant changes in plasma after ethinyl estradiol administration, but these can occur even in the absence of VTE, suggesting additional, undefined mechanisms provoke thrombosis. It is unknown whether endothelial cells (ECs), normally designed to prevent inappropriate initiation of coagulation, are directly altered by OC hormones.

Methods: Human umbilical vein or dermal microvascular endothelial cells (HUVEC and HDMVEC, respectively) were treated with ethinyl estradiol and/or drospirenone, simulating 4th generation OC exposure. TNFα was used as a pro-inflammatory control. EC gene expression was assessed by RT-qPCR; protein expression was measured by western blot. Thrombin generation and fibrin formation were detected via automated calibrated thrombography and spectrophotometry, respectively.

Results: OC hormones did not change EC expression of genes encoding coagulation or fibrinolytic mediators. OC hormones also did not alter EC-supported thrombin generation in platelet-poor plasma or platelet-rich plasma, or in the presence of exogenous tissue factor. Accordingly, OC hormones did not alter fibrin formation.

Meagan **Bridges** **PITM** **Toxicology and Environmental Medicine**

Tobacco Product Use Results in Nasal Immune Mediator Production Most Consistent with M1-like Macrophage Phenotype

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The use of tobacco products, such as cigarettes and e-cigarettes, have been shown to impair respiratory immune cell function. In particular, respiratory macrophages play a critical role in mediating both innate and adaptive immunity, and their function in vitro has been shown to be altered with cigarette smoke and e-cigarette aerosol exposure. While macrophage function is known to be altered, the impact of tobacco product exposure on airway macrophage phenotype is not well understood. As macrophage function is partially linked to phenotype, this is an important knowledge gap to fill. Thus, this research was conducted to examine the impact of tobacco product use on changes in markers of macrophage phenotype (M1 and M2). For this study, we collected nasal lavage fluid (NLF) and serum samples from healthy human subjects who self-identified as non-smokers, cigarette smokers, and e-cigarette users. We evaluated these samples via enzyme-linked immunosorbent assay (ELISA) and Real-time Polymerase Chain Reaction (RT qPCR). Protein markers included CCL17, CCL18, MMP-2 and MMP-9, and gene expression markers included NOS2 and PTGS2 . Data were analyzed using one-way ANOVA with a post hoc Tukey test performed by GraphPad PRISM v 9.3.1. Significance of protein and mRNA levels were set to P<0.05. NLF MMP-9 levels were significantly lower in cigarette smokers compared to non-smokers. Additionally, there was an increase in NLF PTGS2 expression in E-cigarette users compared to non-smokers. There was no significant difference in NLF or Serum CCL17, CCL18 or MMP-2 levels amongst the Tobacco Groups. As reductions in MMP-9 and increases in PTGS2 have previously been associated with M1-like macrophage phenotypes, these results suggest that tobacco product use may result in more predominant M1-like macrophages in the airway compared to non-smoker controls.

Johnny **Castillo** **PITM** **Pathology and Translational Sciences**

SGK3 controls the expression of innate immunity regulators by inhibiting NDRG1 in macrophages.

Johnny Castillo, Robert Hagan3, and Albert S. Baldwin

The innate immune system is essential in the detection and early response to viral infection. For example, double stranded DNA (dsDNA) viruses activate the cGAS/STING pathway, which increases the production of interferon beta (IFNβ) via TBK1/IRF3/7 signaling. In turn, IFNβ promotes the activation of the JAK-STAT pathway, leading to the upregulation of immune-related genes aimed at eliminating the virus. A serine/threonine kinase called serum and glucocorticoid kinase 1 (SGK1) has been implicated in innate immunity yet, its role in the cGAS/STING pathway activation is largely unknown. We demonstrated that 14h, an inhibitor of the SGK family (SGK1/3) prevents STING, TBK1, and IRF3 phosphorylation; IFN production; and STAT3 activation in a dose-dependent manner after treatment with DMXAA, a STING agonist, in Raw264.7 macrophage cells and mouse bone marrow derived macrophages (BMDM). To validate these results, we generated CRISPR-mediated individual or double knock out of SGK1 and SGK3 Raw264.7 cells. We found that SGK1 and SGK3 KO has no effect on phosphorylation of STING, TBK1 or IRF3 after DMXAA treatment. Interestingly, we observed a significant reduction at the protein and mRNA levels of innate immune regulators IRF1, IRF7, IRF9, STAT1, STAT2, IFN and on the levels of STAT3 phosphorylation in SGK3, but not SGK1 KO cells. To describe the mechanism by which SGK3 is regulating the expression of these regulators, we performed a knockdown (KD) of NDRG1, a known SGK3 substrate, in SGK1/SGK3 KO cells. NDRG1 KD rescued the expression of all the aforementioned innate immune regulators. Future studies will address how SGK3-NDRG1 pathway controls the expression of innate immunity regulators in macrophages.

Yogitha **Chareddy** **PITM** **Genetics and Molecular Biology**

Determining the therapeutic potential of dual suppression of c-Myc and KRAS

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Despite the established use of small-molecule inhibitors (SMIs) to target disease-causing proteins, “undruggable” proteins still comprise 70-85% of the proteome and present a significant clinical challenge. The GTPase KRAS and transcription factor c-Myc comprise two of the most prevalent “undruggable” oncoproteins. Aberrant expression of c-Myc and a constitutively active mutant form of KRAS have been implicated in several aggressive cancer types with high mortality rates, including lung and pancreatic cancer. Traditional pharmacological approaches have failed to produce SMIs directly targeting c-Myc and KRAS in cancer cells due to factors such as inaccessible localization and lack of enzymatic binding sites. An SMI targeting a glycine-to-cysteine mutation at codon 12 (G12C) of KRAS has reached the clinic, but this mutation accounts for only ~11% of all KRAS mutations. Interestingly, previous work has revealed mutant KRAS and c-Myc signaling are highly coupled in cancer progression.

RNA interference (RNAi) presents an attractive therapeutic option that is specific and dosage-dependent for “undruggable” proteins. RNAi uses small interfering RNA (siRNA) molecules to bind to and induce degradation of messenger RNA, leading to transcriptional silencing. Recent innovation in the RNAi field has conferred drug-like properties to siRNAs that address previous barriers to clinical application. My project harnesses RNAi to assess the therapeutic potential of dual suppression of c-Myc and KRAS in cancer cells.

Rachel **Cooke** **PITM** **Chemistry**

Synthesis and Assembly of Biohybrid Polymer Libraries

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Compositionally diverse block copolymers have numerous applications in a variety of fields, including drug delivery and protein stabilization. Despite their promising versatility of use, the synthesis of block copolymers remains challenging: being a time intensive process along with problems of incorporating various monomer classes. Our platform uses RAFT polymerization to generate a large library of compositionally diverse polymer blocks and leverages the specificity of DNA hybridization to guide their assembly. Through the synthesis of DNA-polymer conjugates using a wide variety of traditional incompatible monomer classes, such as acrylamides, methacrylates, and acrylates, we can generate a diverse set of block copolymers in fewer synthetic steps than most traditional approaches to block copolymer synthesis. Assembly of these conjugates in aqueous conditions is accomplished using a DNA annealing method, similar to those used in PCR protocols. Once assembled, these block copolymers are analyzed using size exclusion chromatography and gel electrophoresis. This platform will be tested for use as a stabilizing agent for protein-based therapeutics that suffer from storage and degradation issues. In contrast to many current methods for polymer-based stabilization of proteins, such as PEGylation, our platform will not require the chemical modification of the protein. Through these assays we anticipate a greater understanding of how structure function relationships in block copolymers correlate to properties that are useful for the stabilization of protein-based therapeutics.

Arianna **Dame Cascone** **PITM** **Neuroscience Curriculum**

Data analysis plan: Brain-iron neurophysiology and its relationship to the effects of dopaminergic modulation on response inhibition in children with

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Children with ADHD exhibit impairments in response inhibition that have been linked to dopaminergic dysfunction. Dopaminergic modulation ameliorates these impairments via administration of both rewards and dopamine (DA) agonists like methylphenidate (MPH). It is currently unclear, however, whether DA availability is related to the effects of dopaminergic modulation on response inhibition. Thus, we will assess whether variability in basal ganglia tissue iron, an indirect measure of DA availability, is related to responsivity to dopaminergic modulation. To examine this, 66 medication-naïve children with and without ADHD (8-12y) underwent fMRI scans and completed standard and rewarded response inhibition tasks. Children with ADHD participated in a double-blind, randomized, placebo-controlled, crossover MPH challenge. MR-based assessments of brain tissue iron will be used to examine relationships between tissue iron and response inhibition performance. In both groups, we will assess whether variability in tissue iron predicts responsivity to rewards. In the ADHD group, we will also assess whether variability in tissue iron predicts responsivity to MPH with and without rewards. Linear regressions controlling for age and sex will relate tissue iron to response inhibition performance in all analyses. Statistical tests in each analysis will be FDR-corrected at p<0.05, separately for each group. We hypothesize that individuals with greater brain tissue iron levels will exhibit better response inhibition and greater performance improvements following the administration of rewards and MPH, separately and in combination. The proposed study will clarify the role of basal ganglia DA in the cognitive effects of dopaminergic modulation in children with and without ADHD.

Coral **del Mar Alicea Pauneto** **CCBTP** **Pharmacology**

Characterizing myeloid-directed immunotherapy effect in SHH-medulloblastoma

Coral Del Mar Alicea Pauneto (1,2), Duhyeong Hwang (3), Timothy R. Gershon (2,4,5),Marina Sokolsky (3)

We present a novel immunotherapy for medulloblastoma (MB), the most common malignant pediatric brain tumor. Standard therapy in medulloblastoma fails 20% of patients and produces disabling neurotoxicities in survivors. Targeted therapies may improve outcomes by reducing off-target effects and toxicity to healthy tissues. Immune-mediated therapies have revolutionized treatment for many cancers, including melanoma and renal carcinomas, and may be an effective approach to targeted therapy for medulloblastoma. Our prior single-cell RNA sequencing (scRNAseq) studies in both patient samples and mouse models showed that SHH medulloblastomas are heterogeneous communities of cells, in which multiple subsets of myeloid cells comprise 10% of the total population. Prior studies have identified markers for myeloid subpopulations that promote medulloblastoma growth, like IGF1, and in contrast, CCR2-expressing myeloid cells inhibit tumor progression. In medulloblastoma, different myeloid populations have been shown to exert tumor-promoting and tumor-suppressive effects.

My preliminary data show that myeloid-directed therapy can slow tumor growth in mice genetically engineered to develop medulloblastoma. Diverse myeloid cells express Toll-like receptor 7 (TLR7), inducing inflammatory responses when activativate. I found that treating medulloblastoma-prone mice with a nanoparticle formulation of the TLR7/8 agonist resiquimod (POx-resiquimod) increases tumor myeloid populations, slows tumor growth, and increases event-free survival (EFS) compared to mice with untreated tumors. I hypothesize that POx-resiquimod immunotherapy slows medulloblastoma progression by repolarizing tumor-suppressive myeloid cells in the TME. I will study the mechanisms of POx-resumed efficacy and resistance in medulloblastoma to optimize myeloid immunotherapy for future translation to the bedside.

Cherise	**Glodowski**	**CCBTP**	**Pathobiology and Translational Science**

Single cell RNA-sequencing identifies intra-tumoral cellular heterogeneity and drug-induced subpopulation shifts in TNBC Mouse Models

Cherise R. Glodowski (1,2) Kevin R. Mott (2), Denis Okumu (3), Michael P. East (3), Tim C. Elston (3), Gary L. Johnson (2,3), Charles M. Perou (1,2,3,4)
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Triple Negative Breast Cancer (TNBC) is an aggressive malignancy with a poor prognosis accounting for 10-20% of breast cancer cases worldwide. It is thought that intra-tumoral heterogeneity and tumor cell plasticity contribute to drug resistance in TNBCs. This work aims to identify the genetic regulators of plasticity between subpopulations of TNBC cells and to test whether drugs can block or initiate this plasticity to increase chemo-sensitivity. We hypothesize that tumor cell populations shift cell states into drug-resistant populations in response to treatment. To test this, we are treating TP53-/- Genetically Engineered Mouse Model (GEMMs) syngeneic transplant tumors with targeted agents such as the MEK inhibitor trametinib, a chromatin remodeling inhibitor I-BET151, and chemotherapeutics carboplatin and paclitaxel. To examine the cellular subpopulations response to treatment, we performed both in vivo and in vitro drug sensitivity testing, as well as gene expression profiling of single cell RNA-sequencing (scRNA-seq). Thus far, we have identified clear intra-tumor heterogeneity with distinct cell subpopulations including basal, mesenchymal, and proliferative subpopulations in vivo. Preliminary scRNA-seq on single-agent treated tumors shows changes in these cell subpopulation frequencies compared to untreated tumors. Treatment with MEK inhibitor, I-BET151, and carbotax shows the rise of a rare basal-like subpopulation, and another rise in an equally rare stem-like cell population. Gene signatures generated from this stem-like population predict poor patient outcomes in human TNBC datasets. Ultimately, identifying the genetic regulators of these subpopulation changes and targeting these expanded rare cell types should lead to improved therapeutic regimens for patients with therapy-resistant TNBCs.

Anna **Goddard** **CCBTP** **Pathobiology and Translational Science**

Cross Talk: Triple Negative Breast Cancer Tumorigenesis and the Immune Response

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Triple negative breast cancer (TNBC) poses a therapeutic challenge because it lacks expression of targetable estrogen, progesterone, and HER2 receptors. TNBC is characterized by high levels of genome instability and an immunosuppressed phenotype, which can drive therapeutic resistance and metastatic progression. How TNBC adapts to tolerate chronically high levels of genome instability and evade immune clearance is poorly understood. There is a critical role for meiotic recombination 11 (Mre11) in mediating the tumor suppressive response to DNA double-stranded breaks generated during oncogenic hyperplasia. Mre11 deficiency accelerates tumorigenesis, resulting in tumors with high levels of genome instability and immune cell suppression. My project will elucidate the relative contributions of tumor cell-intrinsic Mre11, innate, and adaptive immune sensing pathways to genome instability and immune suppression during breast tumorigenesis. I will introduce lentivirus encoding Cre recombinase and control, Mre11, cGas, or B2M targeting small guide RNA (sgRNA) via mammary intraductal injection into our mouse model. Hyperplastic mammary glands will be harvested monthly and processed for flow cytometry. Histopathological analyses will also be used to characterize the immune profile to early neoplasia induced by these gene mutations. Optimization of mammary gland isolation and dissociation is necessary to increase cell viability and staining for flow cytometry.

Priya **Hibshman** **CCBTP** **Cell Biology & Physiology**

Defining the role of MYC in KRAS-mutant pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer-related deaths in the United States, and current standards of care for PDAC are limited to ineffective cytotoxic chemotherapy. One promising direction for targeted therapies is the KRAS oncogene, mutationally activated in 95% of PDAC. In 2021, the first direct KRAS inhibitor was approved for KRAS-mutant lung cancer. However, initially responsive patients relapse due to treatment-induced resistance mechanisms. A majority of these resistance mechanisms involve alterations in signaling components that drive reactivation of the key KRAS effector pathway, the RAF-MEK-ERK mitogen-activated protein kinase (MAPK) cascade. Despite the critical role of ERK MAPK signaling in PDAC, the crucial ERK targets mediating ERK-driven PDAC growth remain largely undefined. Substantial evidence supports the MYC oncoprotein as a key ERK substrate, but the mechanistic contribution of MYC to KRAS-driven PDAC remains poorly understood. To address this question, my studies have taken two complementary approaches. First, I evaluated the signaling and cellular consequences of KRAS versus MYC suppression. These analyses indicated that KRAS and MYC phenocopy and drive many overlapping cellular processes. Second, I applied RNA-Seq analyses to determine the MYC-dependent versus KRAS-dependent transcriptome. My detailed analyses reveal an important role for MYC in driving KRAS-dependent metabolic processes. In summary, my studies identify diverse KRAS-driven cellular activities facilitated by MYC while also highlighting alternative KRAS-driven mechanisms independent of MYC.

Becky **Hirsch** **PITM** **CBP**

Defining the role of CD73 in β-catenin mutant endometrial cancer for precision medicine

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Most endometrial cancer (EC) cases are diagnosed at an early stage and cured by surgery alone. A gap in knowledge is that 20% of these patients recur, do poorly, and biomarkers to predict recurrence are lacking. While missense mutations in exon 3 of CTNNB1, the gene encoding β-catenin, identify patients at higher risk, not all patients recur. We previously reported that ecto-5'nucleotidase (CD73) downregulation in EC with exon 3 mutant β-catenin is a strong predictor of recurrence. Here, using a 4-site (S33A, S37A, T41A, S45A) exon 3 Xenopus β-catenin mutant, we show by cellular fractionation and co-IP that CD73 limits nuclear translocation of mutant β-catenin by sequestering it at the membrane with E-cadherin. In EC, single site mutations in exon 3 are common. To interrogate the oncogenic nature of patient-relevant β-catenin mutations in the presence and absence of CD73, we identified common exon 3 CTNNB1 mutated residues in EC, using the Cancer Genome Atlas (TCGA), and developed patient-specific β-catenin mutants (D32N, S33F, S33Y, S37C, and S45F) using site-directed mutagenesis. S33F, S33Y, and S37C correspond to phosphorylation sites for glycogen synthase kinase-3β (GSK3β), and S45 is phosphorylated by casein kinase (CK). Characterization of the mutants in EC cancer cells (HEC-1-A) demonstrated decreased or complete absence of phosphorylation at these residues. Further characterization studies with CD73 knockdown have yielded early results demonstrating different oncogenic effects of patient-specific β-catenin mutants. These preliminary data suggest that loss of CD73 is a major oncogenic regulator of β-catenin mutant endometrial tumors.

Maddy **Jenner** **CCBTP** **Pharmacology**

Establishing a co-culture system of pancreatic tumor-stroma cells

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Pancreatic ductal adenocarcinoma (PDAC) remains a highly lethal disease and has a treatment success rate of less than 10%. Therefore, more effective therapies are urgently needed. The major cell type in the PDAC microenvironment are cancer-associated fibroblasts (CAFs) which can shield the tumor from chemotherapy, secrete pro-tumorigenic factors, and produce matrices that contribute to a dense, fibrous microenvironment. Traditional ways of developing drugs for PDAC involve screens against tumor cells but ignore the important interplay between PDAC cells and CAFs. The goal of my research is to find kinases, or drug targets, for PDAC that are influenced by CAFs. My hypothesis is that the presence of CAFs in a co-culture setting can change the drug sensitivity of PDAC cells. I will be using patient-derived xenograft (PDX) PDAC cell and organoids in co-culture with CAFs to more closely resemble the PDAC microenvironment. PDAC and CAF cultures will be infected with different types of luciferase enzymes so that their viabilities can be determined using a luminescence-based system. The co-cultures will be treated with an annotated kinase inhibitor library that was curated based on our PDX proteomics screen. I will identify hits that have a quantified drug response to both pancreatic tumor cells and CAFs and evaluate how the response changes in co-culture. My research studies PDAC in an innovative way and will find kinase targets and kinase inhibitors that target CAF-supportive pathways.

Lauren **Kass** **PITM** **Pharmaceutical Science - DPMP**

Leveraging Continuous Liquid Interface Production (CLIP) to Design Scaffolds for Controlled Stem Cell Delivery

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Glioblastoma (GBM) is the most common form of brain cancer, with an average of only 15 months survival after diagnosis. GBM is highly invasive, and cannot be eliminated with surgery alone. Moreover, chemotherapy and/or radiotherapy treatments are ineffective and highly toxic, ultimately resulting in tumor recurrence. Thus, new methods for targeting and eliminating post-surgical GBM lesions must be developed.

Neural stem cells (NSCs) have emerged as a promising treatment strategy for GBM patients. NSCs exhibit the ability to migrate towards tumor cells via cytokine signaling pathways. NSCs that have been engineered to secrete tumor-toxic payloads (tNSCs) may then be implanted in the brain to seek out and destroy any remaining tumor cells after surgical removal of the main tumor mass. However, tNSCs implanted directly into the GBM resection cavity show a persistence of only 2 weeks in mice, thus limiting the therapeutic durability of the tNSCs.

Biomaterials can be used to encapsulate tNSCs to prevent their rapid clearance. We found that three-dimensional (3D) matrices extend persistence over 6-fold compared to direct-injection or other matrices. Despite these promising results, further optimization is required to advance this much needed tNSC/scaffold therapy forward for use in a clinical setting.

Aditi **Kothari** **PITM** **Pharmacology**

NF-κB and NRF2 pathways dysregulation is associated with improved outcomes in HPV-associated head and neck cancer

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Despite the increasing epidemic of HPV+ HNSCC, the mechanisms of HPV-driven carcinogenesis in head and neck cancers have not been thoroughly investigated. Due to the relatively favorable prognosis of HPV+ HNSCC, along with severe side-effects seen in patients due to radiotherapy and a high level of treatment related morbidity, it is pertinent to develop de-intensification strategies. Using molecular characteristics of HPV+ HNSCC and based on the presence or absence of NF-κB activating mutations, two intrinsic subtypes of HPV+ HNSCCs have been identified. This subtype harboring mutations in key NF-κB inhibitors- TRAF3 and CYLD, is associated with activated NF-κB, maintenance of episomal HPV, and improved patient survival. All this suggested that these tumors were driven by a distinct mechanism of oncogenesis that is dependent on episomal HPV and NF-κB activity. An interesting finding from the study was that these tumors with increased NF-κB activity had a reduced NRF2 signaling. NRF2 has been associated with resistance to treatment and this reduction in NRF2 activity may be one possible explanation of improved patient survival. Preliminary data suggests an inverse correlation between NF-κB and NRF2, with NF-κB potentially altering the oxidative stress response and conferring radio sensitivity to HPV+ HNSCC with TRAF3/CYLD mutations. This project uncovers the role of NF-κB in head and neck cancer etiology which could help identify potential treatments for this subset of HPV associated head and neck tumors while improving quality of life in patients and ultimately contribute to therapeutic personalization.

Rhianna **Lee** **PITM** **Cell Biology & Physiology**

Robust W1282X-CFTR rescue by a small molecule GSPT1 degrader

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With the approval of Trikafta, the vast majority of people with cystic fibrosis (CF) are eligible for CFTR modulator therapy. Remaining individuals have premature termination codons or rare CFTR variants with limited treatment options. Although clinical modulator response can reliably be predicted using primary airway epithelial cells, primary cells carrying rare CFTR variants are scarce. To overcome this obstacle, these cells can be expanded by overexpression of mouse Bmi-1 and human TERT (hTERT). We therefore used this approach to develop two non-CF and three CF (F508del/F508del, F508del/S492F, W1282X/W1282X) nasal cell lines and two W1282X/W1282X bronchial cell lines. Bmi-1/hTERT cell lines recapitulated primary cell morphology and ion transport function and predicted therapeutic responses to CFTR modulators. The F508del/F508del and F508del/S492F cell lines robustly responded to CFTR modulators, which was mirrored in the parent primary cells and the cell donors' clinical response to Trikafta. CC-90009, a novel cereblon E3 ligase modulator targeting the GSPT1 protein, rescued ~20% of wildtype CFTR function in our panel of W1282X/W1282X cell lines and primary cells. Intriguingly, CC-90009 also diminished epithelial sodium channel function. These studies demonstrate that Bmi-1/hTERT cell lines faithfully mirror primary cell responses to CFTR modulators and illustrate novel therapeutic approaches for the W1282X CFTR variant.

Michelle **Mac** **PITM** **Microbiology and Immunology**

SETD2: Setting the the stage for H3K36 Trimethylation in Epigenetic Regulation of HPV Life Cycle

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High-risk types of human papillomavirus (HPV) are associated with multiple human cancers, including cervical cancer and an increasing number of head and neck cancers. The HPV genome is histone-associated and subject to epigenetic regulation. SETD2 is the sole methyltransferase in mammals responsible for placing the trimethyl mark on H3K36 (H3K36me3), a mark of active transcription. SETD2-mediated H3K36me3 recruits effector proteins to regulate multiple cellular processes, including homologous recombination (HR) repair and alternative splicing, which are also important during the HPV life cycle. In previous studies, we defined a critical role for SETD2-mediated H3K36me3 in the life cycle of high-risk HPV31, with SETD2 depletion leading to a loss of H3K36me3 on viral chromatin and a defect in productive viral replication. We previously showed that HR repair factors, including Rad51, bind to HPV chromatin and are required for productive replication. However, the mechanism of how Rad51 is recruited to the viral chromatin is unclear. SETD2 promotes HR repair of double strand breaks (DSB) in actively transcribed genes through the recruitment of LEDGF to H3K36me3. In response to DNA damage, LEDGF recruits CtIP, which promotes resection of DSBs and the recruitment of Rad5. Using chromatin immunoprecipitation (CHIP) coupled with qPCR, we have found that SETD2 depletion leads to an increase in gH2AX, a marker of DSBs, on viral chromatin coincident with a decrease in Rad51 binding, suggesting H3K36me3 protects viral genome integrity. Additionally, we have found that LEDGF and CTIP are bound to HPV chromatin in a SETD2-dependent manner, with CtIP specifically recruited to viral chromatin during productive replication. Furthermore, we have found that LEDGF is required for productive viral replication. Overall, these studies suggest that the enrichment of H3K36me3 on transcriptionally active viral genes promotes the rapid repair of amplifying viral DNA through the LEDGF-CtIP-Rad51 axis.

Breanna	**Mann**	**PITM**	**Pharmacoengineering and Molecular Pharmaceutics**

An organotypic tissue platform to bridge in vitro and in vivo assays for brain cancer treatment

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Brain cancers remain one of the greatest medical challenges. The lack of experimentally tractable models that recapitulate brain structure/function represents a major impediment. Platforms that enable functional testing in high-fidelity models are urgently needed to accelerate the identification and translation of therapies to improve outcomes for patients suffering from brain cancer. In vitro assays are often too simple and artificial while in vivo studies can be time-intensive and complicated. Our live, organotypic brain slice platform can be used to seed and grow brain cancer cell lines, allowing us to bridge the existing gap in models. These tumors can rapidly establish within the brain slice microenvironment, and morphologic features of the tumor can be seen within a short period of time. The growth, migration, and treatment dynamics of tumors seen on the slices recapitulate what is observed in vivo yet is missed by in vitro models. Additionally, the brain slice platform allows for the dual seeding of different cell lines to simulate characteristics of heterogeneous tumors. Furthermore, live brain slices with embedded tumor can be generated from tumor-bearing mice. This method allows us to quantify tumor burden more effectively and allows for treatment and retreatment of the slices to understand treatment response and resistance that may occur in vivo. This brain slice platform lays the groundwork for a new clinically relevant preclinical model which provides physiologically relevant answers in a short amount of time leading to an acceleration of therapeutic translation.

Carmen **Marable** **PITM** **Neuroscience**

Social Determinants Modify the Relationship between Neonatal Inflammation and Brain Volume Later in Life

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While neonatal inflammation is tied to adverse neurodevelopmental outcomes later in life, relationships between inflammation and brain volume in adolescence are understudied. Additionally, the influence of socioeconomic status (SES) on the relationship between neonatal inflammation and reduced brain volumes has not been well characterized. To fill this gap, we used data from the Extremely Low Gestational Age Newborn (ELGAN) cohort, to investigate the relationship between neonatal inflammation measured during the first few weeks of life and brain volume measured at age 15. From the originally enrolled cohort born extremely preterm (23 to 27 weeks' gestation), we examined a sub-cohort of 190 children with paired neonatal inflammatory protein and adolescent MRI data. Elevated blood concentrations of 6 inflammatory proteins (e.g., C-reactive protein) in the first few weeks of life were measured. As an index of SES, we used a composite score based on mother's health insurance, marital status, education, and food stamps. We hypothesized that there would be an association between neonatal inflammation and reduced total brain volumes in adolescents and that this effect will be modified by SES. Neonates who displayed moderate levels of sustained neonatal inflammation (e.g., 2-3 inflammatory proteins) had reduced total brain volume, (δ - 49; p = 0.014). When stratified by SES risk score at birth, in the high SES group (n=128), the inflammation-associated brain volume reduction remained significant (δ -58; p = 0.038), but in the low SES group (n=62), this association was not found (δ -24; p = 0.47). In conclusion, among individuals born extremely preterm, neonatal systemic inflammation was associated with decreased brain volume in adolescence, but this association was not found among individuals with indicators of social disadvantage.

Syed **Masood** **PITM** **Curriculum in Toxicology and Environmental Medicine**

Live Cell Imaging of Oxidative Stress in Human Airway Epithelial Cells Exposed to a Secondary Organic Aerosol

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Exposure to air pollution is the leading cause of global morbidity and mortality. Specifically, exposure to fine particulate matter (PM2.5) is known to cause pathological complications such as the onset of respiratory disease and premature death. The largest source of PM2.5 is from photooxidation of isoprene, the most abundant non-methane hydrocarbon in the atmosphere, to isoprene hydroxy hydroperoxide (ISOPOOH). ISOPOOH leads to the formation of isoprene-derived secondary organic aerosols (SOA). Previous research has shown isoprene-derived SOA exposure in human airway epithelial cells (HAEC) induces increased expression of inflammatory and oxidative stress gene markers. Furthermore, it has been demonstrated that isoprene-derived SOA can generated reactive oxygen species and exposure to HAEC leads to activation of Nrf-2. However, the molecular initiating event of ISOPOOH exposure remains unknown, thus our hypothesis is ISOPOOH exposure of HAEC leads to glutathione oxidation potentially through lipid peroxidation. Our experimental approach relies on live-cell imaging of HAEC expressing roGFP, a genetically encoded fluorogenic sensor that specifically reports on the changes in the intracellular glutathione redox potential. Micromolar exposure of HAEC to ISOPOOH induced a rapid and robust glutathione oxidation independent of the generation of hydrogen peroxide suggesting ISOPOOH initiates lipid peroxidation. Total lipid extracts of ISOPOOH-exposed HAEC had oxidative potency suggesting lipid peroxidation of cellular membranes. Furthermore, supplementation of with HAEC with poly-unsaturated fatty acids also exacerbated ISOPOOH-induced glutathione oxidation. These findings demonstrate that ISOPOOH is potent and unique environmental peroxide that contributes to the oxidative burden posed by exposure of SOA by initiating lipid peroxidation of cellular membranes.

Minna **McFarland** **PITM** **Neuroscience**

Sex and acute, but not chronic, cocaine administration alters allopregnanolone levels in specific regions of rat brain

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Neurosteroids are compounds synthesized within the brain that influence neuronal activity, in part, via actions at gamma-aminobutyric acid (GABA) type A receptors. The endogenous GABAergic steroid (3a,5a)-3-hydroxy-5-pregnan-20-one, or allopregnanolone, a metabolite of progesterone, has emerged as a clinically beneficial therapeutic for the treatment of psychiatric disorders, such as post-partum depression and substance use disorders. Moreover, multiple psychiatric disorders are associated with altered brain and serum levels of neurosteroids. Clinically, chronic cocaine use was correlated with decreased levels of pregnenolone, a precursor to allopregnanolone, and increasing allopregnanolone levels via progesterone administration in humans with a history of cocaine use resulted in reduced cocaine craving and stress arousal. We predicted that allopregnanolone levels would increase after acute cocaine, but after prolonged (chronic) cocaine exposure, allopregnanolone levels would decrease compared to controls. Therefore to test the effect of acute and chronic cocaine on allopregnanolone levels, we performed two separate studies to test how systemic administration of 15mg/kg (1) acute (40-minutes) or (2) chronic cocaine (14 days) affects brain (olfactory tubercle, frontal cortex, dorsal striatum, midbrain) and serum allopregnanolone levels in adult male and female rats. Results indicate that while cocaine acutely increases allopregnanolone levels in the midbrain (14% increase in males; 40% increase in females), chronic cocaine exposure does not persistently increase allopregnanolone levels in any region in either sex. Interestingly, we found that allopregnanolone levels also varied by sex across brain regions, with females exhibiting significantly increased levels of allopregnanolone in the olfactory tubercle, frontal cortex, and in serum, but not midbrain. Collectively these results suggest allopregnanolone levels vary across brain regions and by sex, which may play a part in sex differential responses to stress.

Sophie **Mendell** **PITM** **Pharmaceutical Sciences - DPMP**

Development of CAR T Cells Targeting CD70

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CD70 is increasingly being investigated as a potential target for cell-based cancer immunotherapy. CD70 expression is upregulated in several cancers, including acute myeloid leukemia and glioblastoma, with transient expression patterns in a subset of tissues under healthy conditions. In this talk I will discuss the design and optimization of chimeric antigen receptor T cells targeting CD70. This will include discussion on ongoing construct optimization as well as our current in vitro results.

Ryan **Mouery** **CCBTP** **GMB**

Investigating Cross-Talk Between Kinase and Ubiquitin Signaling in the Cancer Cell Cycle

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Due to their role in controlling cellular proliferation and their frequent contribution to tumorigenesis, members of the cell cycle machinery are often considered attractive therapeutic targets. However, many targets currently undergoing clinical investigation are considered “pan-essential” resulting in a low therapeutic index and limited efficaciousness. For example, due to its oncogenic potential, extensive effort has gone into developing small-molecule inhibitors targeting the polo-like kinase 1 (Plk1), yet most drug candidates have faced challenges in clinical trials due to dose-limiting toxicities. Recent work, however, has led to the successful use of the Plk1 inhibitor Onvansertib for the treatment of KRAS-mutant metastatic colorectal cancer. Here, we have utilized various approaches to further characterize Plk1-mediated signaling networks in an attempt to improve the efficacy of Plk1-targeted therapies. In doing so, we have found that Plk1 activity contributes to the abundance and stability of the E3 ubiquitin ligase Cyclin F, a protein involved in regulating cell cycle progression and maintenance of genome stability. In response to Plk1 inhibition, Cyclin F abundance is decreased and, consequently, the abundance of many Cyclin F substrates is increased. Significantly, we have found that Cyclin F loss is more pronounced in colorectal cancer cell lines harboring a KRAS mutation when compared to an isogenic cell line containing wild-type KRAS and this results in the differential accumulation of Cyclin F substrates. These results may uncover novel vulnerabilities in KRAS-mutant colorectal cancer cells that may be targeted concurrently with Plk1 to improve the efficaciousness of this therapeutic approach.

Brandon **Mouery** **CCBTP** **Genetics and Molecular Biology**

CDK4/6 inhibition induces an RB-dependent downregulation of the minichromosome maintenance (MCM) complex

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Inhibitors of cyclin-dependent kinases 4 and 6 (CDK4/6) have emerged as promising breast cancer therapeutics. Unfortunately, dose-limiting toxicities and intrinsic and acquired resistance remain clinical barriers. A better understanding of the mechanism of action of CDK4/6 inhibitors is thus needed. CDK4/6 inhibitors function primarily by preventing inactivation of the retinoblastoma protein (RB), thus repressing E2F-mediated gene expression and inducing a G1 arrest. However, little is known about other molecular changes induced by CDK4/6 inhibitors. Using 3 CDK4/6 inhibitors as well as p16 overexpression, we have discovered that CDK4/6 inhibition leads to proteasome-dependent loss of the minichromosome maintenance (MCM) complex proteins in an epithelial cell line. MCM is an essential DNA replication factor, and its dysregulation can result in replication stress, DNA damage, and cancer. To ensure genome stability, the chromatin localization of MCM is highly regulated throughout the cell cycle. Conversely, MCM abundance remains steady in cycling cells. We found that CDK4/6i-induced MCM downregulation is not simply a consequence of repressed transcription because an ectopic MCM2 protein driven by a constitutive promoter is still downregulated in response to CDK4/6 inhibitors. We believe this observation represents the first known mechanism regulating MCM abundance through active protein degradation. Furthermore, we have found that RB depletion abrogates CDK4/6i-induced MCM downregulation, indicating an RB-dependent phenotype. Given that MCM is essential for DNA replication, we speculate that CDK4/6i-induced MCM downregulation may limit the proliferative capacity of both cancer and normal cells and may be an important mechanism by which CDK4/6 inhibitors exert their anti-proliferative effects.

Mariaelena **Nabors** **CCBTP** **Pharmacology**

Growth Media Influences PDAC Subtype

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Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death in the U.S. with a dismal five-year survival rate of 11%. PDAC has two prognostically relevant transcriptional subtypes, termed classical and basal-like. Basal-like tumors are more aggressive while classical tumors have a better prognosis and are more sensitive to chemotherapy. There is a growing consensus in the field that the PDAC subtypes are not permanently encoded but rather exist in a plastic state. It is unclear if shifts in subtype are caused by changes in specific signaling pathways, physical factors such as the extracellular matrix, or crosstalk with the stroma. Our lab has observed shifts in subtype in some of our 2D patient derived xenograft cell lines when grown in different media conditions. To investigate this observed plasticity we grew patient-derived xenograft (PDX) cell lines in different media conditions and did RNA-sequencing to identify potential pathways involved in regulating PDAC transcriptional subtypes.

Dina **O'Connell** **CCBTP** **Pathobiology & Translational Sciences**

Role of MHC Class I Antigen Presentation in Triple-Negative Breast Tumor Metastasis

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Triple-negative breast cancer (TNBC) has a poor prognosis that worsens dramatically after distant metastasis. Clinical data from paired primary breast tumors and metastases demonstrates that TNBC metastases frequently lose expression of genes involved in major histocompatibility complex class I (MHC-I) antigen presentation, including human leukocyte antigen A (HLA-A). All nucleated cells in the body present endogenous antigenic peptides on their cell surface to cytotoxic T cells using MHC-I proteins. Tumors with high mutational burden can elicit an adaptive immune response through neoantigen presentation, or conversely, evade detection by decreasing MHC-I pathway activity. We hypothesize that HLA-A loss increases metastatic potential by causing immune evasion through decreased MHC-I antigen presentation. CRISPR/Cas9-mediated gene knockouts of H2-K, the mouse ortholog of HLA-A, were generated in the mouse mammary tumor cell line KPB25Luv. Protein-level validation confirms successful knockout of H2-K while retaining H2-D expression. Transcriptomic profiling through cDNA-based microarrays identified a gene signature of H2-K knockout that correlates with poor survival and decreased expression of MHC-I genes in human breast tumor samples. Following this in vitro characterization, H2-K knockout cell lines will be injected into the tail vein of immunocompetent mice to evaluate the effect of H2-K knockout on metastatic formation. This work will provide direct evidence of the importance of MHC-I pathway activity in TNBC progression, with the long-term goal of overcoming this immune-evasive phenotype to improve patient outcomes.

Carli **Opland** **PITM** **Neuroscience**

Caspase-dependent tau cleavage as a pathogenic mechanism in Alzheimer's Disease

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With an estimated 5.8 million Americans affected, Alzheimer's disease (AD) is a neurodegenerative disease associated with extracellular amyloid-beta plaques and intracellular accumulation of tau protein leading to pathological neurofibrillary tangles. Normally, in a healthy individual, tau is a microtubule-associated protein that stabilizes axonal microtubules in neurons. However, in an AD diseased neuron, tau collapses into twisted strands to form tangles correlated with neuronal degeneration. Tau undergoes many different post-translational modifications (PTMs), however, these modifications are still poorly understood on how they contribute to tau pathogenesis in AD. Tau cleavage by caspases is believed to be an early toxic modification that precedes other later pathological modifications and drive neuronal degeneration found in human AD brain. With few reliable model systems with which to study this process, our lab has discovered that proteasome defects result in caspase-3 activation resulting in robust tau cleavage. In the absence of caspase-3 in neurons, tau cleavage is no longer produced suggesting that caspase-3 is the main component driving tau cleavage. As it is still unclear where and how tau is cleaved in the neuron, I observed both cleaved tau and caspase-3 enrichment at the post-synaptic density (PSD) where toxic tau species may be causing further damage resulting in neuronal network dysfunction. Our study suggests that proteasome deficiency and subsequent accumulation of cleaved tau is a pathogenic mechanism that incites neurodegeneration in AD.

Macy **Osborne** **PITM** **Pathobiology and Translational Science**

Investigating the Role of RPL22 in MSI-high Endometrial Cancer

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Endometrial cancer (EC) is rising in both incidence and mortality each year. EC can be divided into four distinct molecular subtypes. The MSI-high subtype is characterized by insertions/deletions in microsatellite regions due to the loss of expression of DNA mismatch repair (MMR) proteins. Microsatellite instability results in the acquisition of mutations that can act as neoantigens for immune system recognition. Therefore, we would expect to see large numbers of tumor infiltrating lymphocytes (TILs) in MSI-high ECs. However, preliminary data has demonstrated a large variation in the number of TILs in the MSI-high EC subtype. It is not well-understood how immune cells are recruited and why such a large deviation in the number of TILs exists in MSI-high ECs. Recently, RPL22 has been identified as being mutated in approximately 52% of MSI-high ECs. Further, 86% of these mutations are truncating which result in loss of functional RPL22 protein. RPL22 loss has been shown to alter translation efficiency of mRNA, leading to reduced protein synthesis. I hypothesize that RPL22 loss in MSI-high EC directly affects immune cell activation and recognition of tumor cells by altering translation of MHC-class I receptors. To test this hypothesis, I will use patient samples to characterize RPL22 expression in relation to TILs along with cell-based experiments to determine the role of RPL22 in MHC-class I receptor translation and immune cell activation in MSI-high ECs. This work is of significant translational relevance because it will give insight into the molecular events that regulate TIL recruitment and activation.

Cassandra **Phillips** **PITM** **Cell Biology & Physiology**

Effects of Osmotic Stress on Intermediate Filament Dynamics and Regulation in Disease

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Intermediate filaments (IFs) are cytoskeletal proteins critical for cellular stress responses. Mutations in IF and IF regulatory genes cause more than 70 human diseases that commonly exhibit abnormal IF accumulation and aggregation. Giant Axonal Neuropathy (GAN) is a fatal pediatric neurodegenerative disease characterized by abnormal IF dynamics and pathologic accumulation and aggregation, resulting in a loss of sensory and motor function. GAN is caused by loss-of-function mutations in the gene KLHL16, which encodes an E3 ubiquitin ligase adaptor (gigaxonin) responsible for IF degradation. My central hypothesis is that KLHL16 mutations compromise IF cytoskeleton stress responses and interfere with cellular recovery from stress. Brief hypotonic stress exposure was shown to cause rapid reorganization and degradation of vimentin IFs, which led us to interrogate this mechanism in the context of GAN. We demonstrated that GAN patient and control fibroblasts exhibited time-dependent vimentin cleavage and network breakdown in response to hypotonic stress, and that this was significantly elevated in GAN cells. Since calpain enzymes are known to be involved in IF regulation, we further probed this pathway. Total expression of calpain proteins was increased in GAN cells compared to control cells, and pharmacologic inhibition of calpains reduced vimentin cleavage. We observed striking colocalization between large perinuclear vimentin aggregates and calpain-2 in GAN cells, which was altered by hypotonic stress. Finally, mass spectrometry proteomics revealed regulatory PTM sites at Ser-409/412 on vimentin involved in this stress response. Future experiments will focus on advancing mechanistic understanding of stress-associated IF proteostasis mechanisms in GAN.

Ryan **Robb** **CCBTP** **Pharmacology**

Elucidation of the interplay between autophagy and macropinocytosis in ERK MAPK inhibited pancreatic cancer

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Alteration of essential metabolic pathways is a major mechanism by which oncogenic KRAS (KRAS*) promotes tumor development and growth in pancreatic ductal adenocarcinoma (PDAC). KRAS*-driven PDAC is dependent on nutrient scavenging pathways, including macropinocytosis (MP) and autophagy to fuel the high metabolic demand of rapid proliferation. Thus, these metabolic processes are attractive targets for the development of treatments for PDAC. KRAS loss results in downregulation of MP in KRAS*-driven PDAC. Additionally, my lab demonstrated that inhibition of ERK MAPK signaling decreased glucose consumption and glycolysis but increased autophagy, thereby enhancing dependency on autophagy for survival and growth. Accordingly, dual ERK MAPK and autophagy inhibition synergistically enhanced anti-tumor efficacy in KRAS*-driven PDAC. While preclinical results showed promise, early clinical data has demonstrated that resistance to this treatment arises over time through unknown mechanisms. My preliminary data indicates that following ERK MAPK inhibition both autophagy induction, and MP downregulation, can only be sustained transiently—with autophagy and MP activity returning to/surpassing basal levels after prolonged treatment. The underlying mechanistic and signaling crosstalk between autophagy and macropinocytosis remains poorly understood. I hypothesize that there is a compensatory regulation between autophagy and MP signaling following ERK MAPK inhibition through which prolonged activation of autophagy upregulates macropinocytosis over time, consequently abrogating dependency on autophagy. My study is focused on elucidating the compensatory relationship by which prolonged activation of autophagy induces upregulation of MP. A better understanding of the signaling underlying these metabolic resistance pathways will inform future ERK MAPK inhibitor combinations.

Jack	**Sanford**	**CCBTP**	**Genetics & Molecular Biology**

Cytoplasmic p53 binds lactate dehydrogenase (LDH) B and regulates LDH activity in a transcription-independent manner

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p53 suppresses tumorigenesis via a concerted set of functions that alter the cell cycle, cell death, metabolism, and DNA damage repair. Although several studies have identified cytoplasmic, transcription-independent functions of p53, the relevance of cytoplasmic functions of p53 has not been elucidated, particularly in an in vivo context. Here, we generated the p53K316P mutant mouse model, which mimics a naturally occurring p53 nuclear localization signal (NLS) amino acid change observed in bat species. We found that p53K316P mutation increases cytoplasmic localization of p53 and promotes a pleiotropic metabolic phenotype that includes increased adiposity, increased de novo lipogenesis, and decreased lactate generation. These phenotypes occur in the absence of detectable alterations in p53 target gene expression in mouse embryonic fibroblasts and mouse tissues, suggesting that the phenotypes may occur through transcription-independent activities of p53. Studies using transcriptionally inactive p53K316P variants revealed that p53K316P is able to suppress lactate production in the absence of target gene transactivation. Immunoprecipitation (IP)-mass spectrometry revealed that p53 binds lactate dehydrogenase (LDH) B, a cytoplasmic enzyme that interconverts pyruvate and lactate. We show that p53K316P is able to alter LDH composition and activity in such a manner that favors pyruvate generation and hinders lactate production, consistent with the increased adiposity and decreased lactate production of the p53K316P mouse.

Stephen **Serafin** **CCBTP** **Cell Biology & Physiology**

Elucidating the RAMP protein interactome that drives tumor lymphatic growth.

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Tumor metastasis is a significant cause of cancer-related morbidity and mortality and commonly occurs through invasion of cancerous cells into the lymphatic system. Tumor-associated (TA) lymphangiogenesis, which is the growth of the lymphatic system in response to the tumor microenvironment, actively contributes to tumor metastasis and remains poorly understood. Thus, there is a need to characterize key regulators of lymphangiogenesis under healthy and pathological conditions. A therapeutically tractable class of proteins implicated in lymphangiogenesis are G-protein coupled receptors (GPCRs) and their associated receptor-activity modifying proteins (RAMPs). RAMPs allosterically regulate GPCR trafficking, signaling, and recycling. The potent pro-lymphangiogenic and tumor-secreted peptide, adrenomedullin (AM), promotes TA-lymphangiogenesis and tumor metastasis. The AM receptor consists of the heterodimerization of the GPCR calcitonin receptor-like receptor (CLR/Calcrl) with either RAMP2 or RAMP3. The goal of this project is to characterize the mechanism by which RAMPs regulates CLR trafficking and intracellular signaling in lymphatic endothelial cells (LECs) and how this contributes to the process of lymphangiogenesis. Here, I utilize proximity dependent biotin identification (miniTurbo) coupled to mass spectrometry to identify members of the AM- and CLR-dependent RAMP interactomes to determine the mechanism by which RAMPs regulate CLR. Importantly, how RAMPs regulate GPCR function and physiology is still largely unknown, thus unearthing the RAMP interactomes will help to identify mechanisms of regulation. Elucidating the role of RAMPs in CLR pharmacology and physiology will lay the foundation for a new era of RAMP-targeted drug development, which has exciting implications for disease.

Chelsea **Smith** **PITM** **Pathobiology and Translational Medicine**

The Role of Polymerase Theta in DSB Repair and Resistance to Breast Cancer Therapies

Chelsea M. Smith (1,2) Wanjuan Feng, PhD (2), Gaorav Gupta, MD, PhD (2)

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DNA Polymerase Theta (Polq) has emerged as an exciting potential target for breast cancer treatment, and drugs recently entered phase 1 clinical trials. This follows the discovery that Pol θ is upregulated in some cancers and correlates with worse prognosis. Pol θ is a conserved protein involved in the DNA double strand break (DSB) repair pathway, Theta-Mediated End Joining (TMEJ). Sacituzumab govitecan (SG), an antibody-drug conjugate with a topoisomerase I (TOPOI) inhibitor (SN-38) payload, has recently been shown to improve survival of metastatic triple negative breast cancer. However, resistance to SG is a significant hurdle. Genetic studies have demonstrated that Polq KO cells are more sensitive to TOPO1 inhibitors. I have shown that Polq KO sensitizes cells to SN-38. I plan to further this finding by analyzing foci formation of Polq, DNA damage markers, and Rad51, a key protein involved in the high-fidelity DSB repair pathway homologous recombination (HR), after treatment. How a cell decides between HR or TMEJ repair remains unknown. Additionally, I plan to utilize the technique CUT&RUN to uncover specific DNA sequences of Pol θ recruitment after DNA damage. These findings will further the understanding of the complex process of DSB repair pathway choice, as well as explore the potential of Pol θ inhibition in combination therapies. In addition, by better understanding regions of Pol θ recruitment, this can contribute towards biomarker development for tumors that could benefit from Pol θ targeting therapies.

Markia	**Smith**	**PITM**	**Pathobiology and Translational Science**

DNA Damage repair classifier defines distinct groups in hepatocellular carcinoma

Markia A. Smith (1), Sarah C. Van Alsten (2), Jeffrey S. Damrauer (3), Cyrus Vaziri (1), Ugwuji N. Maduekwe (4), Michael I. Love (5,6), Melissa A. Troester (1,2,3), Katherine A. Hoadley (3,5)

The prognosis of patients with hepatocellular carcinoma (HCC) remains poor, with a 5-year survival rate of 18%. While advancements have been made in pathophysiology and molecular characterization of HCC, a more robust and reproducible gene signature could provide a better understanding of the role of DNA repair in liver cancer. We curated panel of 199 DNA repair pathway genes representing fifteen DNA repair pathways. Leveraging The Cancer Genome Atlas (TCGA) HCC study (n=374), we assessed DNA repair expression patterns to provide a better understanding of the crucial role DNA repair plays in liver carcinogenesis and progression. We evaluated associations with clinicopathologic variables and risk factors by DNA repair classes. We developed a dynamic bimodal distribution RNA-based DNA repair signature that identified two groups in HCC based on DNA repair gene expression: Low repair and High repair. The Low repair group had increased expression of classical HCC tumor markers (ALB, HNFA), enriched in CTNNB1 mutations and lower grade tumors. High repair tumors showed high DNA repair activity, high grade, HBV positive status, and increased frequency of p53 mutant-like and TP53 mutation status. Liver regeneration and mitosis signatures were higher in High repair than the Low repair. Differences in p53 status underscores the importance of p53 in liver tissue homeostasis and tumorigenesis. High repair tumors have worse progression-free (HR 1.64, p=0.001) and overall (HR 1.64, p=0.006) survival compared to the Low repair group. Repair high and low HCC tumors have distinct biological features and provides a rationale for developing DNA-targeted therapies.

Colleen **Steward** **CCBTP** **Microbiology and Immunology**

Unraveling B cell differentiation in pancreatic cancer using engineered neoantigens

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Pancreatic cancer is the third leading cause of cancer-related mortality and a growing public health burden. Late-stage diagnosis and poor response to existing treatments, including immunotherapy, contribute to a bleak 5-year survival rate of 11%. There is, therefore, a need to develop combination therapies and harness immune cell populations within the tumor microenvironment. B cells represent a prominent population of infiltrating immune cells in pancreatic cancer and have the capacity to elicit both anti- and pro-tumorigenic responses. Effector B cells facilitate anti-tumor immunity, whereas regulatory B cells support tumor growth. B cell differentiation is influenced by the affinity of antigens to the B cell receptor, yet it is unclear how antigen affinity and localization impact B cell responses within the tumor microenvironment. To address these questions, we engineered a system to model varying antigen affinity and compare antigens expressed on the cell surface to intracellular antigens. In the model, hen-egg lysozyme (HEL) with low, intermediate, or high affinity for the B cell receptor in transgenic MD4 mice is either secreted or expressed on the surface of murine pancreatic cancer cells. Given that the isolation of B cell specific tumor antigens in vivo has yet to be streamlined, our system enables us to control antigen localization, dictate antigen affinity, and observe the resulting B cell response. Understanding the mechanisms by which tumor antigens shape B cell responses in cancer will help inform the design of B cell-directed immunotherapies to enhance anti-tumor immunity.

Michael **Sturdivant** **CCBTP** **Pharmacology**

APOBEC3 Induced Mutagenesis in Urothelial Carcinoma

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As the most common malignancy of the urinary tract among men and women, bladder cancer is estimated to surpass 80,000 new cases and 17,000 deaths is 2022. Accounting for most bladder cancers cases, urothelial carcinoma has a dismal survival rate of 5% in the metastatic setting. APOBEC3A (A3A) and APOBEC3B (A3B) are members of a family of cytidine deaminase enzymes that catalyze the removal of an amino group from cytosine nucleotides generating a uracil in its place that serve as a source of mutations. A3A and A3B enzymes are commonly overexpressed in urothelial carcinoma with the APOBEC mutation signature seen in most cases. Due to their mutagenic activity, A3A and A3B have been implicated in altering the genomic landscape of urothelial carcinoma tumors over time. These alterations have the potential to augment tumor cell clonality that can lead to an enrichment of cells that are refractory to treatment and drive disease progression. Currently, it is unclear if both A3A and A3B drive APOBEC-induced mutagenesis, or if one enzyme plays a larger mutagenic role than the other. To address this question a mouse cancer cell line entitled BBN963, that is representative of human muscle invasive bladder cancer, has been developed to express either A3A or A3B in the presence of doxycycline. Utilization of this model will allow for a direct comparison of A3A and A3B induced mutagenic activity, tumor cell clonality diversification, and modulation of response to treatment.

Taylor	**Tibbs**	**PITM**	**Microbiology & Immunology**

Genetics determine susceptibility to viral hemorrhagic fever

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Viral hemorrhagic disease leads to severe organ failure, perfuse bleeding, and often death. Mortality rates can vary widely in infected individuals, from 25-90%. The biological mechanisms that drive these differences in disease outcome remain unknown. Animal models have been critical tools for studying human disease. An inbred line of laboratory mice, called FVB, develop a hemorrhagic disease that mimics human symptoms when infected with lymphocytic choriomeningitis virus (LCMV). My data show that FVB susceptibility is heritable and immune-mediated. I hypothesize that hemorrhagic disease in LCMV-infected FVB mice is due to genetic deviations that adversely affect the immune response to viral infection. Through forward genetic breeding and bioinformatic analysis, I have identified a 25 megabase region on Chromosome 17 linked to viral disease in FVB mice. Furthermore, I have discovered that disease in FVB appears to be driven by both innate and adaptive immune mechanisms. Identification of specific genetic and immune determinants in mice may reveal comparable targets in humans; therefore, allowing the development of better diagnostic tools and therapeutics for viral hemorrhagic disease.

Tamara **Vital** **CCBTP** **Not Available**

Title not available Not Available

Abstract Not Available

Sara **Wasserman** **PITM** **Pharmaceutical Science--Chemical Biology and Medicinal Chemistry**

Chemical Epigenetic Modifier-Mediated Dose Control of Gene Therapy

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Advances in delivery systems for gene therapy such as recombinant Adeno-Associated Virus (rAAV) have enabled the development of treatments that correct the underlying cause of genetic diseases. However, there is currently not a translatable method by which the expression of gene therapy transgenes can be controlled. As many genetic diseases involve dose-specific biological processes, the development of a titratable system of dose control could improve the safety and efficacy of gene therapy. This project optimizes Chemical Epigenetic Modifier (CEM) technology to achieve dose control of rAAV-based gene therapy. CEMs are bifunctional small molecules that facilitate recruitment of endogenous epigenetic machinery to targeted genetic loci through association with synthetic DNA binding proteins such as zinc fingers. CEM-mediated recruitment of the transcriptional upregulator, bromodomain-containing protein 4 (BRD4), increases expression of rAAV transgenes in a dose-dependent manner. Ongoing efforts will also utilize CEM technology to directly modulate genomic expression of disease-relevant loci. The development of a translatable CEM system will provide a novel mechanism of control that may improve gene therapy outcomes.

Alex **Woodell** **PITM** **Not Available**

Engineered human induced-neurospheres enhance CAR-T therapy for glioblastoma Not Available

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