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# Cancer Research Day

## 2022 Abstract Book

# POSTER #1

## TELOTRISTAT ETHYL, A SEROTONIN BIOSYNTHESIS INHIBITOR, ENHANCES STANDARD CYTOTOXIC THERAPY RESPONSE IN CHOLANGIOCARCINOMA

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**Objective:**

Cholangiocarcinoma (CCA) is a highly aggressive biliary tract cancer (BTC) that originates from intrahepatic (10-20%), perihilar (50-60%) or distal (20-30%) bile ducts. CCA has a very poor prognosis with a 5-year survival rate of 5-15%. The majority of CCA patients (~80%) present with unresectable disease where the combination of gemcitabine plus cisplatin (GemCis) is the standard treatment with a median survival of 14 months. This low survival rate warrants the evaluation of novel therapeutic strategies to improve the overall survival of CCA patients. Increased accumulation and secretion of serotonin have been reported to support oncogenic activity in CCA. Telotristat ethyl (TE) is an inhibitor of tryptophan hydroxylase 1 (TPH1) that mediates serotonin biosynthesis. We investigated the therapeutic efficacy of TE in combination with standard chemotherapies in preclinical models of CCA.

**Methods:**

Tumor growth inhibition studies were performed in cell-derived subcutaneous xenografts using human intrahepatic CCA (iCCA) CCLP-1 cells, distal CCA (dCCA) TFK-1 cells, and perihilar CCA (pCCA) SNU-1196 cells in NOD/SCID mice. Tumor growth inhibition studies in patient-derived xenografts (PDX) were performed in NSG mice. Ten days after tumor cell injection, mice were randomized (n=4-7) to receive PBS (control), TE, gemcitabine, cisplatin or *nab*-paclitaxel (NPT) for two weeks. Tumor size was measured twice per week using calipers. Animal survival studies were performed in peritoneal dissemination xenografts in NOD/SCID mice using iCCA CCLP-1 cells. Intratumoral mechanism of action was determined by immunohistochemistry (IHC) and immunoblotting.

**Results:**

Animal survival was substantially improved by NPT (60%), while TE (11%) or GemCis (9%) had a marginal effect. Interestingly, animal survival was extended by the combination of TE to GemCis (26%) and to NPT (68%). In iCCA CCLP-1 xenografts, TE showed 53% inhibition in tumor growth, and NPT (69%) caused greater inhibition than GemCis (53%). In dCCA TFK-1 xenografts, the reduction in tumor growth by TE was 51%, and NPT (56%) was more effective than GemCis (37%). In pCCA SNU-1196 xenografts, tumor growth inhibition by TE, NPT and GemCis was 41%, 67% and 58%. In all three cell-derived xenograft studies, TE combination with chemotherapy demonstrated an improved tumor growth inhibition effect (range: 67-90%). In PDX studies, TE markedly inhibited tumor growth (range: 40-73%), and GemCis caused a greater reduction (range: 80-86%) than NPT (57-76%). Again, an additive effect was observed with the combination of TE and chemotherapy. Effects of TE, GemCis and NPT on tumor cell proliferation (Ki67 staining) corresponded with subcutaneous tumor growth inhibition data. IHC analysis exhibited decreased levels of serotonin by TE treatment in subcutaneous tumors.

**Conclusion:**

TE exhibited marked antitumor efficacy in various CCA xenografts, and it enhanced GemCis or NPT chemotherapy response. Thus, combination regimens with TE have the potential to improve clinical cytotoxic CCA therapy.

***Basic Science Faculty***

# POSTER #2

## SINGLE NUCLEI CHROMATIN ACCESSIBILITY AND TRANSCRIPTOMIC MAP OF BREAST TISSUES OF WOMEN OF DIVERSE GENETIC ANCESTRY

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Single nuclei analysis is allowing robust classification of cell types in an organ that helps to establish relationships between cell-type specific gene expression and chromatin accessibility status of gene regulatory regions. Using the institutional resource of breast tissues of healthy donors of various genetic ancestry, we have developed a comprehensive chromatin accessibility (snATAC-seq) and gene expression (snRNA-seq) atlas of human breast tissues. Our analyses included 51,367 nuclei with average sequence coverage of 1195 genes per nuclei. These nuclei were derived from 22 donors of Ashkenazi descent, 20 of European non-Ashkenazi ancestry, 10 of Asian ancestry, 10 Hispanic/Latina, 10 Native American, 6 from BRCA1 mutation carriers, and 5 from BRCA2 mutation carriers. Although tissues from 20 women of African Ancestry were included in sequencing, poor quality sequencing reads prevented inclusion of data from those samples in the final analysis. Integrated analysis revealed 10 distinct cell types in the healthy breast, which included three major epithelial cell subtypes (mature luminal, luminal progenitor, basal), two endothelial subtypes, two adipocyte subtypes, fibroblasts, T-cells, and macrophages. Mature luminal cells could be further divided into two distinct hormone sensitive subtypes, HSa and HSb, with HSa subtype expressing higher levels of Estrogen Receptor alpha (ESR1) and NEK10, a tyrosine kinase that controls p53 activity and limits cell proliferation. The luminal progenitors could be broadly classified into alveolar progenitors (AP) and basal-luminal hybrid progenitors (BL). AP cells expressed higher levels of ELF5, a known marker of alveolar cells, compared to BL cells. Basal cells could be classified into two subtypes with Basal-BAa cells being the most dominant and expressing higher levels of TP63 and NFIB compared to Basal-BAb cells. ESR1 expression pattern was distinctly different in tissues from Native Americans compared to the rest, with a high level of ESR1 expression extending to AP cells. In fact, overall AP cell numbers were ~3-fold higher in Native Americans compared to others (18.9% versus 1.5-8.8%). Furthermore, Ingenuity pathway analysis of differentially expressed genes revealed elevated Estrogen Receptor signaling in ML and AP cell types of Native Americans compared to those of other genetic ancestry. Despite significant differences in expression and activity in Native Americans compared to others, the chromatin accessibility map of the ESR1 gene regulatory regions in ML and LP cells did not show any genetic ancestry dependent variability. In general, cell subtype-specific gene expression did not correlate with chromatin accessibility differences, suggesting that transcriptional regulation independent of chromatin accessibility governs cell type-specific gene expression in the breast. Collectively, these results reveal complexities in gene expression in different cell types of the breast.

***Basic Science*** ***Faculty***

# POSTER #3

## EVALUATION OF REPLICATION PROTEIN A INHIBITOR IN COMBINATION WITH EGFR MUTANT TARGETED THERAPY

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Lung cancer is the leading cause of cancer related deaths worldwide with the majority (~80%) of patients diagnosed with non-small lung cancer (NSCLC). Targeted therapies directed against tumor initiating growth factor receptors are effective treatment options for driver mutation positive NSCLC. Osimertinib is a third-generation EGFR-Tyrosine Kinase inhibitor (TKI) that has activity against EGFR exon 19, exon 21, and T790M mutations. Unfortunately, Osimertinib treated patients acquire resistance and eventually succumb to disease progression. The acquisition of secondary mutations leading to activation of bypass pathways, MET amplification, EMT, compensatory pathways and histological transformation to small cell phenotype are major causes of acquiring resistance to EGFR TKIs. Proliferating cancer cells regularly experience a low level of replication stress. Additionally, receptor tyrosine kinases such as EGFR are known to interact with DNA repair proteins and impact DNA damage repair following chemotherapy, radiation therapy, and EGFR TKI treatment. However, the involvement of DNA repair pathways in EGFR-TKI resistance is unknown and the interaction between EGFR pathway, DNA damage, and repair pathways have not been fully elucidated. At low-level replicative stress promotes genomic instability but at a high level through mitotic catastrophe causes cell death. Replication protein A is a critical sensor of the DNA damage response detecting replication stress. The small molecule inhibitor of RPA, **NERx 329**, sequesters active RPA and induces replication catastrophe and cell death. Here we demonstrate that **NERx329** enhanced Osimertinib mediated cell death when added in combination. CCk-8 assays performed after 48h of **NERx329** and Osimertinib combination treatment result in a robust decrease in $IC_{50}$ compared to single agent therapy. Dissection of signaling pathways leading to this death promoting effect of **NERx329** showed inhibition of bypass and compensatory pathways associated with acquired resistance. From these preliminary data, we infer that DNA damage repair pathways could be involved in TKI resistance and **NERx329** could be a promising drug candidate for combination targeted therapy.

***Basic Science*** ***Faculty***

# POSTER #4

## TARGETING FGF21 INHIBITS TUMOR GROWTH AND ATTENUATES CACHEXIA IN EXPERIMENTAL COLORECTAL CANCER

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**Background:** Colorectal cancer (CRC) is frequently accompanied by cachexia, an uncured multi-organ wasting syndrome, debilitating musculoskeletal health, physical function, and overall survival. Fibroblast growth factor 21 (FGF21) is a modulator of musculoskeletal health and metabolism and has been associated with CRC risk and progression. Here, we investigated the effects of tumor derived FGF21 on musculoskeletal health in CRC. **Methods:** Plasma from CRC patients and preclinical models of CRC (C26, MC38) were assessed for circulating FGF21 levels, and CRC cell lines (C26, MC38, HCT116) were screened for FGF21 expression. Using CRISPR/Cas9 technology, FGF21 was deleted from MC38 cells (KO-MC38). 8-week-old male C57BL/6J mice were subcutaneously injected ($1.0x10^6$) with wild-type (WT-MC38) or $FGF21^{-/-}$ (KO-MC38) MC38 tumor cells, while experimental control animals received saline (n = 10-13/group). Animals underwent electrophysiological testing to obtain motor unit number estimation (MUNE) 48 hours prior to euthanasia and were assessed for maximum plantarflexion torque 24 hours prior to euthanasia. Blood, tumors, skeletal muscles, and bones were collected for additional analyses. **Results:** CRC patients and MC38 tumor hosts demonstrated elevated circulating plasma FGF21 ($p<0.05$) compared to controls. Gene expression of CRC cells revealed an 11-fold increase of FGF21 in MC38 compared to C26 and HCT116 cells. WT-MC38 tumor hosts had elevated plasma FGF21 (4-fold; $p<0.05$) compared to control, which was unchanged in KO-MC38 hosts. In line with the development of cachexia, WT-MC38 hosts displayed reductions in muscle mass (gastrocnemius: -5%; $p<0.05$, quadriceps: -10%; $p<0.01$, tibialis anterior: -8%; $p<0.01$), MUNE (-31%; $p<0.01$), and plantarflexion torque (-10%; $p<0.05$), as well as in trabecular bone volume fraction (BV/TV: -30%; $p<0.05$) and cortical cross-sectional thickness (Cs.Th: -14%; $p<0.05$). Conversely, when compared to WT-MC38, KO-MC38 hosts had preserved skeletal muscle mass (gastrocnemius: +7%; $p<0.01$, quadriceps: +13%; $p<0.001$, tibialis anterior: +8%; $p<0.001$), MUNE (+47%; $p<0.01$), plantarflexion torque (+8%; $p<0.05$), and bone mass (BV/TV: +69%; $p<0.001$, Cs.Th: +23%; $p<0.001$). Strikingly, the mass of KO-MC38 tumors was reduced 75% ($p<0.01$) compared to WT-MC38 tumors. **Conclusion**: Our data suggest that targeting CRC-derived FGF21 halts tumor growth, resulting in preservation of musculoskeletal health and function. Thus, counteracting tumor-derived FGF21 may serve as a therapeutic intervention against the progression of cancer and cancer-associated cachexia.

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# POSTER #5

## DELETION OF FNDC5/IRISIN PROTECTS AGAINST CANCER INDUCED CACHEXIA SYNDROME

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Cancer cachexia (CC) is characterized by severe skeletal muscle and metabolic abnormalities. As the browning of white adipose tissue is a feature of CC and as the hormone irisin has been shown to promote thermogenic energy conversion in adipocytes, we sought to determine if deletion of the precursor for irisin, Fibronectin type III domain-containing protein 5 (FNDC5), could improve CC. Irisin is a circulating hormone cleaved from FNDC5 in response to exercise.

FNDC5 KO animals were implanted with Lewis Lung Carcinoma (LLC) or metastatic MC38 colorectal cancer (mMC38). Our data show that male FNDC5 KO mice are protected against CC induced by both tumors. In contrast, no significant protective effects were observed in the female KO mice. Male FNDC5 KO tumor hosts maintained their normal body weight and skeletal muscle mass in spite of tumor growth in contrast to wildtype (WT) control mice carrying the same tumor mass. Moreover, the deletion of FNDC5/irisin protected against muscle weakness and increased total locomotor activity. Tumor secreted humoral factors have been shown to activate and elevate pro-atrophic pathways in cachectic skeletal muscle, such as STAT3 phosphorylation and *Atrogin1* and *Murf1* expression, all important regulators of protein catabolism. Surprisingly, these regulators were unchanged in the skeletal muscle of the LLC-bearing FNDC5 KO mice compared to non-tumor bearing mice. In addition, metabolic alterations such as increased levels of pyruvate dehydrogenase kinase 4 (PDK4) and succinate dehydrogenase (SDH) activity were unchanged in the skeletal muscle of tumor bearing KO mice compared to non-tumor bearers.

These observations suggest that counteraction of FNDC5/irisin protects against cancer-induced muscle wasting and weakness in a sex dependent manner. Our findings suggest that FNDC5/irisin could represent a novel target for the treatment and prevention of cancer cachexia.

***Basic Science*** ***Faculty***

# POSTER #6

## ERG INFLUENCES CELL FATE DECISIONS

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Prostate cancer is the second most common cancer among men. An estimated 50% prostate cancers harbor a chromosomal rearrangement that causes the *TMPRSS2:ERG* fusion. *TMPRSS2* is a prostate specific, androgen responsive, gene. *ERG* encodes an ETS family transcription factor that has normal roles in hematopoietic stem cells and endothelial cells. Fusion of the promoter and 5' UTR of the *TMRPSS2* gene to the open reading frame of *ERG* results in aberrant expression of either full-length or N-terminally truncated ERG protein in prostate epithelial cells. *ERG* is otherwise silent in adult epithelial cells. The prevalence of this common genetic event makes it attractive as potential therapeutic target. In different models ERG can promote either luminal epithelial fates, or epithelial to mesenchymal transition. We found that TLR4 and VEGF pathways can regulate ERG phosphorylation. Inhibition of TLR4 inhibited ERG phosphorylation and ERG function in a basal cell line, while inhibition of VEGF inhibited ERG phosphorylation and function in a luminal cell line, indicating ERG regulation is cell type dependent. Hence, we hypothesize that, in basal/mesenchymal prostate cancer cells, ERG is activated by TLR4 signaling and functions to promote stemness as in hematopoietic stem cells, and in luminal-epithelial prostate cancer cells ERG is activated by VEGF signaling and functions to promote luminal differentiation similar to the role in endothelial cells. Identification of cell specific ERG regulation is critical for the development of targeted drugs and ensure the translatability of our findings.

***Basic Science*** ***Faculty***

# POSTER #7

## EVALUATION OF THE THERAPEUTIC EFFECTS OF HEPARAN SULFATE MIMETIC IN PANCREATIC DUCTAL ADENOCARCINOMA CACHEXIA

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**BACKGROUND**: Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy with the lowest 5-year survival rate relative to all other solid tumor types and is projected to become the second leading cause of US cancer-related deaths by 2030. PDAC patients frequently develop cachexia, a multifactorial condition with progressive weight loss mainly resulting from reduced skeletal muscle and fat mass. Cachexia has been associated with impaired physical function, poor response rates to chemotherapy and radiotherapy, and increased mortality in PDAC patients. Unfortunately, there is no effective approved therapy for this syndrome. Heparan sulfate (HS) mimetics have been shown to have anti-cancer effects because of their ability to bind and modulate function of many HS-binding proteins important for cancer growth and progression. Given that cancer cachexia is a systemic inflammation-driven syndrome characterized by superinduction of proinflammatory cytokines and many cytokines are capable of binding HS, including those elevated in patients with cancer cachexia and causing muscle and fat wasting, it is surprising that HS mimetics have not been evaluated specifically for cancer cachexia. The aim of this study was to evaluate the therapeutic potential of HS mimetic (HSm) in PDAC cachexia with a particular interest in sex difference of the effect.

**METHODS**: KPC pancreatic cancer cells (5000 per mouse) were injected into the pancreas of 10-week-wild-type C57BL/6 male and female mice to generate orthotopic tumor. The PDAC tumor-bearing mice were treated with the HS mimetic (10 mg/kg; i.p.) at the designated intervals. Body weight and body composition were monitored and tumor and organ tissues were collected. The MTT assay was used to assess the effect of HSm on the KPC cell proliferation.

**RESULTS**: HSm treatment reduced tumor mass in both male and female mice. Body weight loss was observed in both sexes after HSm administration. Interestingly, the loss was gradually reduced over time and eventually there was no difference between the HSm treated and the vehicle control mice. However, male mice recovered more slowly than female mice. Similarly, male mice recovered from the loss of lean and fat mass more slowly than the female mice. Further, skeletal muscle and fat weights at euthanasia showed sex difference; males had more reductions than females in response to the HSm treatment. HSm treatment of KPC cells in culture showed decreased cell proliferation.

**CONCLUSION**: HS mimetic has strong anti-PDAC effect at least partly through inhibition of cancer cell proliferation but reduces body weight and skeletal muscle and fat mass. There is sex difference in the drug effects. Future studies will investigate the mechanisms underlying the HS mimetic inhibition of PDAC cells and the sex difference, which may identify molecular targets for combination therapy to overcome the negative impact of HSm on muscle and fat tissues.

***Basic Science*** ***Faculty***

# POSTER #8

## OXYGEN TENSION - DEPENDENT DIFFERENCES IN CANCER CELL KINOME

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Current approaches to preclinical cancer research often fail to consider the impact of maintaining cancer cells under ambient oxygen ($O_2$) tension (~21%). This is also true for hypoxia studies that typically involves cancer cells previously grown in ambient $O_2$ before subsequent transfer to hypoxic conditions. However, the tumor microenvironment is characterized by significantly lower $O_2$ levels. We have previously demonstrated the impact of ambient $O_2$ tension on stem cell populations, signaling pathways and resistance to therapy. We developed an experimental approach that allows us to collect and process tumor tissues from transgenic mammary tumor mouse models under physioxia (3% $O_2$) such that they are never exposed to ambient $O_2$. In the present study, our goal was to explore kinase signaling pathway alterations that occur due to physioxic and ambient $O_2$ tensions and to determine how these pathways are influenced by treatment with targeted drugs in the context of these $O_2$ levels.

Our studies revealed increased basal phosphorylation levels of EGFR (Y1068) in cells processed and propagated in ambient air (AA), relative to physioxia. However, downstream signaling effectors AKT and ERK showed higher phosphorylation levels under physioxia, compared to AA, suggesting that their activation is independent of EGFR signaling. These findings correlate with the decreased sensitivity of the tumor cells under physioxia to target drugs lapatinib and alpelisib. We then sought to examine basal and target drug induced kinome changes in tumor cells under physioxia and AA via Multiplexed Inhibitor Beads (MIBs) kinome assay. This assay revealed significant differences in the kinome of the tumor cells under physioxia compared to AA. Although direct comparisons between control and lapatinib treated cells under physioxia and ambient air showed very minimal changes, pairwise comparison between lapatinib treated physioxia cells and vehicle treated AA cells revealed an increase in the activity of PDGFRB in lapatinib treated physioxia cells. Similarly, a receptor tyrosine kinase (RTK) array and western blotting showed increased basal and lapatinib induced phosphorylation of PDGFRB (Y751) under physioxia. Next, we determined the potential role of PDGFRB in downstream signaling pathway activation of AKT and ERK and resistance to lapatinib. We found that Sunitinib, a multitarget RTK inhibitor with high affinity for PDGFR effectively decreased PGDFRB activity under physioxia, with a concurrent decrease in the phosphorylation of AKT. Moreover, tumor cells under physioxia were more sensitive to sunitinib treatment, relative to ambient air. Furthermore, a combination of lapatinib and sunitinib rendered tumor cells under physioxia more sensitive to treatment than with lapatinib alone.

These findings suggest that ambient and physioxic oxygen tensions differentially impact cancer relevant signaling pathways. Therefore, it may be necessary to carry out preclinical cancer studies in the context of physiologically relevant oxygen tensions to aid translatability of preclinical cancer studies.

***Basic Science*** ***Graduate Student***

# POSTER #9

## PP2A DRIVES ABERRANT MACROPINOSOME PROCESSING IN PANCREATIC DUCTAL ADENOCARCINOMA.

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Pancreatic Ductal Adenocarcinoma (PDAC) is the fourth leading cause of cancer related deaths in the US, with the lowest five-year survival rate of all cancers.Nutrients in PDAC microenvironment are commonly depleted, with the vital amino acid glutamine among the most deficient metabolites. In an attempt to circumvent this deprivation, PDAC cells initiate KRAS dependent macropinocytosis, an actin-driven nutrient scavenging pathway. The macropinosomes fuse with lysosomes where the constituents are degraded, and the nutrients are recycled. As glutamine is essential for cell survival, therapeutic inhibition of macropinocytosis represents a novel strategy to suppress nutrient acquisition and drive cell death in PDAC.

Protein phosphatase 2A (PP2A) is a heterotrimeric Serine/Threonine phosphatase known to inhibit downstream targets of the KRAS signaling cascade and is implicated in macropinocytosis regulation. PP2A holoenzyme comprises of A, B and C subunits, where the regulatory B subunit provides substrate specificity to the enzyme. B56α has been identified as the regulatory subunit responsible for tumor suppressive function of PP2A through posttranslational modification of oncoprotein, cMYC. We demonstrate that pharmacological activation of PDAC cell lines with the small molecule activator of PP2A-B56α, DT061, leads to significant accumulation of intracellular vesicles. Vesicles formed with DT061 treatment is inhibited by macropinocytosis inhibitor, EIPA and they phenocopy the PIKfyve inhibitor, Apilimod. Therefore, we hypothesize that PP2A activation results in aberrant macropinosome processing by preventing the fusion with lysosomes, leading to vesicle accumulation and PDAC cell death. Using high molecular weight TMR-Dextran, we confirmed that these vesicles with DT061 treatment are macropinosomes. PP2A activation prevented the colocalization of macropinosomes with lysosomes, likely limiting the nutrients supply to PDAC cells. RNA sequencing analysis showed that glutamine deprivation genes are significantly enriched with acute activation of PP2A, and the oxidative phosphorylation pathway was observed to be significantly inhibited. Furthermore, combination of DT061 with Glutamine transport inhibitor V-9302 significantly inhibits the survival of PDAC cell lines.

Together, these findings indicate that activation of PP2A in late stage PDAC promotes aberrant macropinosome processing and establishes a novel role of PP2A in nutrient scavenging and cell death. These pathways can be further exploited to identify potential therapeutics in PDAC.

***Basic Science*** ***Graduate Student***

# POSTER #10

## LYSINE ACETYLATION MODULATES THE ACTIVITY OF NUCLEASES INVOLVED IN LAGGING STRAND DNA REPLICATION

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The well-coordinated action of DNA replication proteins and repair mechanisms maintains the eukaryotic DNA replication machinery. Discrepancies in the genome can trigger genome instability and promote human diseases, including cancer. DNA replication is a semicontinuous process, where the leading strand is replicated continuously while the lagging strand is replicated discontinuously in the form of short DNA segments called Okazaki fragments. Every round of DNA replication generates 50 million Okazaki fragments, each one being initiated by an error-prone, polymerase alpha-synthesized RNA-DNA primer. Since multiple fragments are being generated, processed, and ligated on the lagging strand, there is a high chance of mutagenesis compared to the leading strand. Two redundant pathways are involved in processing Okazaki fragments: the short flap and the long flap. In the short flap pathway, the initiator RNA primer is excised, and the DNA primer synthesized by Pol alpha is ligated. In the long flap pathway, the RNA-DNA primer is removed and resynthesized by high-fidelity polymerase delta. The ability of the cell to select one pathway over the other suggests the presence of a mode of regulation. Diversifying the proteome by post-translationally modifying the enzymes involved in these pathways can serve as a form of regulation.

The Okazaki fragment processing proteins, FEN1 and Dna2, can be modified by p300, a lysine acetyltransferase. Upon lysine acetylation, FEN1 experiences an inhibition in its binding and cleavage activities, while Dna2 experiences a simulation of its binding and cleavage properties. While our studies have utilized OFM intermediates to clarify the effect of acetylation on the nucleases, we aim to decode its impact on their interactive properties. Overall, we propose that lysine acetylation of nucleases improves its activity during DNA transactions in a manner that is consistent with promoting genome stability.

***Basic Science*** ***Graduate Student***

# POSTER #11

## TISSUE-SPECIFICITY OF IL-9R-EXPRESSING TUMOR-ASSOCIATED MACROPHAGES

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The immunosuppressive function of myeloid cells that support tumor progression is controlled by secreted factors found in the tumor microenvironment. Interleukin 9 (IL-9) is a pleiotropic cytokine that signals through the IL-9 receptor (IL-9R) and can function as a positive or negative regulator in tumor immunity. Our recent work demonstrates that IL-9 signaling promotes tumor progression in a B16F10 mouse model of lung metastasis by expanding a CD11c+ interstitial macrophage population and inducing Arginase 1 (ARG1) activity. However, whether this IL-9R+ macrophage population is found in other cancer types remains unknown. Here, we use orthotopic models of breast cancer, colorectal cancer, melanoma, and a spontaneous model of colorectal cancer to identify IL-9R-expressing myeloid cells that contribute to tumorigenesis. Using a 4T1 triple-negative breast cancer (TNBC) model, we identified a population of IL-9R expressing CD11b+ LY6C+ monocytes that are recruited to the tumor microenvironment. Furthermore, we found an IL-9R/ARG1+ macrophage population that is phenotypically similar to the pro-tumorigenic IL-9R/ARG1+ pulmonary macrophage population. In contrast, the spontaneous and orthotopic models of colorectal cancer and melanoma lack the IL-9R+ monocyte and macrophage populations. Thus, our work defines the tissue and tumor tropism of the IL-9R+ monocyte/macrophage populations and provides the basis for additional functional studies.

***Basic Science*** ***Graduate Student***

# POSTER #12

## THE ROLE OF GCN2 KINASE IN LEUKEMOGENESIS AND THERAPEUTIC RESPONSE TO L-ASPARAGINAS

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Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer, affecting the lymphoid cells of both B and T lineages. Most ALL cells are auxotrophic for asparagine, a nonessential amino acid for protein synthesis, due to the low expression of asparagine synthetase (ASNS), a rate limiting enzyme for de novo biosynthesis of asparagine. As a result, standard ALL treatment takes advantage of this vulnerability by giving patients L-asparaginase, a bacterial enzyme that depletes the circulating asparagine. However, previous work from our lab and others have shown that some ALL cells become resistance to L-asparaginase treatment through the induction of ASNS expression. Mechanistically, amino acid starvation activates the general control nonderepressible 2 (GCN2) kinase, leading to the accumulation of ATF4 transcriptional factor. ATF4, in turn, is recruited to the promoter of the ASNS gene to activate its transcription. However, the role of GCN2 kinase in the process of leukemogenesis under nutrient limiting environment has not been established. In this project, our goal is to use a mouse model of T-ALL to determine the role of GCN2 in leukemogenesis and the therapeutic response to L-asparaginase treatment.

***Basic Science*** ***Graduate Student***

# POSTER #13

## GCN2 EIF2 KINASE PROMOTES PROSTATE CANCER BY MAINTAINING AMINO ACID HOMEOSTASIS

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A stress adaptation pathway termed the integrated stress response (ISR) is suggested to be active in many cancers, including prostate cancer (PCa). The ISR features a family of protein kinases that phosphorylate the eukaryotic translation initiation factor 2 (eIF2) during different stress conditions, resulting in repression of global protein synthesis. Paradoxically, eIF2 phosphorylation also enhances the translation of select gene transcripts, such as *ATF4*, which directs the transcription of ISR-target genes that are critical for cancer stress adaptation. Moreover, eIF2 phosphorylation and ATF4 have recently been suggested to play a role in PCa growth and survival; however, the specific function of the ISR kinases, their mode of activation, and the mechanisms by which the ISR facilitate PCa progression are unknown.

We demonstrate that the eIF2 kinase GCN2 is activated in a wide range of PCa cell lines and patient samples, contributing to enhanced eIF2 phosphorylation and ATF4 expression. Inhibition of GCN2 reduced growth in androgen-sensitive and castration-resistant PCa cell lines and cell line-derived and patient-derived xenograft mouse models. Using CRISPR-based phenotypic screens and genome-wide gene expression analyses, we determined that GCN2 is required for expression of genes involved in amino acid synthesis, reclamation, and import. Specifically, GCN2 activation and eIF2 phosphorylation are critical for the maintenance of essential amino acid (EAA) pools in PCa cells by regulating the expression of multiple *SLC*-genes involved in amino acid transport. Inhibition of GCN2 decreased expression of amino acid transporters accompanied by severe depletion of intracellular EAAs, ultimately resulting in amino acid starvation and decreased cell proliferation. We identify that SLC3A2 is an essential SLC gene required for PCa proliferation that is regulated by GCN2. SLC3A2 engages with many nutrient transporters, facilitating their proper localization to the plasma membrane. Of importance, expression of SLC3A2 partially restored amino acid levels and growth due to loss of GCN2 in PCa.

Due to the importance of amino acids in metabolism, we performed untargeted metabolomics on PCa cells treated with GCN2 inhibitor and identified purine and pyrimidine metabolism as key metabolic pathways impacted by loss of GCN2. Supplementation with purines, but not pyrimidines, rescued the growth inhibition due to loss of GCN2 in PCa cells. These results suggest that GCN2 maintenance of amino acids are also contributors to the synthesis of key metabolites, such as nucleotides, that are critical for PCa progression.

We propose that limitation for select amino acids activate GCN2 in PCa, resulting in increased expression of key amino acid transporters, such as SLC3A2, which provide for nutrient import to facilitate protein synthesis and metabolism required for proliferation of PCa. We conclude that PCa requires GCN2 for maintenance of amino acid homeostasis and this eIF2 kinase is a promising therapeutic target for the treatment of PCa.

***Basic Science*** ***Graduate Student***

# POSTER #14

## DEFINING THE MECHANISMS OF TGLI1'S ONCOGENIC FUNCTIONS

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Glioblastoma (GBM) is the most common malignant brain and other central nervous system tumor in adults with a five-year survival rate of less than 7%. Discovered in GBM, tGLI1, a novel alternative splicing isoform of GLI1, is highly expressed in both GBM cell lines and primary specimens but not in normal tissue. Despite an in-frame deletion of 123 base pairs, tGLI1 has retained all known GLI1 functional domains, continues to respond to Sonic Hedgehog signaling, and translocates into the nucleus, similar to GLI1. Moreover, tGLI1 operates as a gain of function transcription factor with the ability to bind to and activate genes unique from GLI1 to promote invasion, migration, angiogenesis, and stemness in both glioblastoma and breast cancer. Despite the advancements in our understanding of tGLI1, a genome wide DNA binding pattern of tGLI1 has not been established and the mechanism by which tGLI1 gains access to these genes is unknown. To establish a more robust understanding of the differential DNA binding patterns of GLI1 and tGLI1, we carried out ChIP-sequencing (ChIP-seq) and found tGLI1 to be significantly enriched at 1313 unique sites while GLI1 was significantly enriched at 1197 unique sites. As only 417 regions were bound by both proteins, we concluded that GLI1 and tGLI1 have distinct DNA binding patterns and show unique binding from one another beyond the previously established genes. Next, to determine whether variations in protein interactions of GLI1 and tGLI1 contribute to the unique binding pattern of tGLI1, we carried out immunoprecipitation (IP) followed by mass spectrometry. Results showed only 8 of 35 proteins are associated with both GLI1 and tGLI1. Based on these results, we concluded GLI1 and tGLI1 have a large set of distinct interacting partners, which suggests GLI1 and tGLI1 are likely to function differently in the cell.

***Basic Science*** ***Graduate Student***

# POSTER #15

## HUNK PHOSPHORYLATES RUBICON TO SUPPORT AUTOPHAGY, PROMOTING TUMORIGENESIS IN HER2+ BREAST CANCER

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Human epidermal growth factor receptor 2-positive (HER2+) breast cancer is defined by having HER2 gene amplification that coincides with HER2 protein overexpression. HER2 is amplified in 15-30% of breast cancers and overexpression of this gene is a predictor of survival in breast cancer patients. HER2-targeted therapies have been successful in treating HER2+ breast cancer; however, HER2+ breast cancer can develop resistance to these therapies establishing an urgent need for novel targets within HER2+ breast cancer to inhibit tumorigenesis. Previous work in our lab establishes that Hormonally Upregulated Neu-associated kinase (HUNK) is a Serine/Threonine (S/T) protein kinase that is overexpressed in HER2+ breast cancer and is responsible for promoting autophagy, thereby leading to therapeutic resistance and tumorigenesis in HER2+ breast cancer. Our previous work shows that HUNK phosphorylates the autophagy protein, Rubicon, at S92 on its' N-terminal domain promoting autophagy in 293T cells. However, we have yet to establish a role for this phosphorylation site within HER2+ breast cancer cells. Therefore, the objective of this study is to identify if this phosphorylation event plays a role in promoting increased tumorigenesis in HER2+ breast cancer. To further elucidate the role that phosphorylation of Rubicon at S92 plays in the underlying mechanisms of tumorigenesis in HER2+ breast cancer, we generated multiple scientific tools: a phospho-specific antibody to detect Rubicon phosphorylation at S92, a phospho-deficient (S92A) Rubicon mutant, and a phospho-mimetic (S92D) Rubicon mutant. We established an autophagy phenotype within a set of cell lines derived from the MMTV-neu mouse model. These cells are derived from MMTV-neu HUNK wild type (WT) or MMTV-neu HUNK knockout (KO) tumors. These cell lines will be utilized alongside our phospho-deficient and phospho–mimetic Rubicon mutants to determine the role that Rubicon S92 phosphorylation plays within HER2+ breast cancer in-vitro and in-vivo.

***Basic Science*** ***Graduate Student***

# POSTER #16

## RESOLVING THE DIFFERENTIAL REGULATION OF ERG AND EWS/FLI1 IN ONCOGENESIS

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ETS transcription factors serve essential roles in development, angiogenesis and hematopoiesis. The ETS family is composed of 28 members that share a highly conserved DNA-binding domain and bind consensus sequences containing a 5'-GGAA-3' core. Chromosomal rearrangement events can lead to the overexpression of specific ETS fusion proteins. Overexpression of ERG occurs in nearly half of all prostate tumors. Our lab has shown that interactions between ERG and EWS are required to promote malignant phenotypes in prostate cancer cell lines. EWS is a ubiquitous RNA-binding protein that also functions as a strong transcriptional coactivator. EWS/ETS fusions have been implicated in Ewing Sarcoma (ES), a less common pediatric cancer. Although the expression of EWS/FLI1 is most prevalent (~95%), fusions involving EWS/ERG have also been identified in patients. ERG and FLI1 are close homologs with nearly 70% amino acid similarity, yet FLI1 does not interact with endogenous EWS nor does it contribute to oncogenic phenotype in prostate cell lines. Given the high degree of sequence identity between their DNA binding domains, the mechanisms by which ERG and EWS/FLI1 specifically contribute to oncogenesis remain unresolved.

Our lab has demonstrated that overexpression of ERG or EWS/FLI1 in RWPE1 epithelial prostate cells lead to increased migration, a phenotype associated with prostate cancer cell models. From these results, I hypothesized that ERG and EWS/FLI1 share genomic binding sites to promote similar transcriptional profiles in ES. I conducted ChIP-sequencing in A673 cells, a ES cell line expressing endogneous EWS/FLI1, with or without expression of exogenous ERG. Surprisingly, ERG and EWS/FLI1 bound a large number of unique sites with minimal peak overlap. Gene ontology analyses suggest that ERG expression may promote a mesenchymal phenotype. Previous groups have shown that knockdown of EWS/FLI1 in A673 cells promotes migration and reduces anchorage-independent survival. Though I have yet to evaluate migration, expression of ERG significantly reduces anchorage-independent survival. I aim to evaluate the mechanisms by which ERG and EWS/FLI1 are differentially regulated to influence gene expression and drive cellular phenotype. I am currently investigating the role of EZH2 in the A673 model system. It has been established that EZH2 interacts with the N-terminus of ERG to regulate its activity. Furthermore, phosphorylation of ERG at S96 disrupts this interaction. In ES tumors, EWS/ERG fusions result in N-terminal truncations of ERG, potentially preventing regulation by EZH2. As a result EWS/ERG may function more similarly to EWS/FLI1 than full length ERG.

***Basic Science*** ***Graduate Student***

# POSTER #17

## FARP1-RAC1 AXIS HAMPERS CD8+ T CELLS-MEDIATED IMMUNOSURVEILLANCE BY REDUCING ANTIGEN PRESENTATION

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Immune evasion plays a critical role in tumorigenesis. Current immune checkpoint therapies show tremendous efficacy by enhancing cytotoxicity of $CD8^+$ cytotoxic T lymphocytes (CTLs). However, cancer cells develop different mechanisms of immune evasion, among which suppression of immune recognition of somatic mutations via tumor antigen is prevalent. Despite the previous assumption that the mutation burden of a tumor is the principal determinant of immunity and immunotherapy response in colorectal cancer (CRC), a recent study identified the expression level of the neoantigens as another key driver of immune evasion. There is, nonetheless, little achievement toward the therapeutic approaches to restore antigen presentation on tumor cells. CRC, the second deadliest cancer in U.S., shows increasing incidence and mortality rates over the past years. Standard conventional CRC treatments, surgery, chemotherapy and radiotherapy, often lead to many side effects due to their non-specificity and cytotoxicity. While immunotherapy is very promising, only a small population of CRC patients respond to the current immune checkpoint inhibitors. Thus, the aim of this study was to harness a novel immunological approach to target CRC tumors. By developing a bioinformatics tool, we created a gene library whose expression negatively correlates with cytotoxicity of $CD8^+$ CTLs in the tumor microenvironment (TME) of CRC patients. Given the central role of antigen presentation in immunity and activating $CD8^+$ CTLs, further biological screening was based on the scope of each gene's capacity to modulate antigen presentation. To this end, we developed stable clones of OVA expressing MC38 cells ($MC38^{OVA}$) where OVA-derived peptide, SIINFEKL, is bound to MHC-I complex for cell surface presentation. Two genes with highest potential to alter MHC-I cell surface levels were identified. Our consequent in vivo experiments along with in vitro cytotoxicity assays and bioinformatics analysis identified FARP1 as the top actionable target with striking suppressive effects on tumor growth. FARP1 knockdown (KD) significantly inhibited tumor growth in immunocompetent mice whereas it showed minor effects on immunocompromised mice, suggesting that FARP1 may modulate tumor growth by regulating the interaction of tumor cells with non-tumor cells such as immune cells. Consistent with the major contribution of antigen presentation in boosting immune surveillance, both $CD8^+$ CTLs activity and infiltration showed a significant increase in the TME of FARP1 KD MC38-derived tumors compared to the wild type tumors. FARP1 is a guanine nucleotide exchange factor (GEF) for Rho GTPases. Our further molecular mechanistic studies showed that RAC1 Rho GTPase is the major downstream effector of FARP1 to modulate antigen presentation. Taken together, we introduced FARP1-RAC1 axis as a potential clinical target to restore immune evasion in CRC tumors.

***Basic Science*** ***Graduate Student***

# POSTER #18

## CANCER ASSOCIATED FIBROBLASTS PROVIDE A CANCER STEM CELL NICHE THAT LEADS TO DISEASE RELAPSE IN OVARIAN CANCER

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Epithelial ovarian cancer is the most lethal gynecologic malignancy with a 5-year survival rate of only 48%. While most ovarian cancer patients respond to chemotherapy initially, frequent relapse (~80%) and development of chemoresistance result in poor patient outcomes. Cancer stem cells (CSCs) consist of a small subpopulation in the tumor that is capable of surviving from chemotherapy and causing tumor relapse. Using patient specimens and in vitro models, we determined that CSCs are enriched by cancer associated fibroblasts (CAFs) after chemotherapy. Cancer associated fibroblasts (CAFs) are a major constituent of the ovarian cancer tumor microenvironment and are highly enriched in the residual tumors following chemotherapy. Therefore, we studied the mechanism by which CAFs promote ovarian cancer chemoresistance and disease relapse. CAFs isolated from ovarian cancer patient tumors were used in heterotypic 2D or 3D coculture systems with high-grade serous ovarian cancer cell lines or with patient-derived ovarian cancer cells to study their effect on CSCs and chemoresistance. Matched pre-and post-chemotherapy patient tumors were used to confirm our findings. CAFs significantly increased adjacent cancer cell resistance to carboplatin by enriching CSCs. Pre-coculture with CAFs increased in vivo tumor initiation capacity of the ovarian cancer cells by 10-fold in limiting dilution assay analysis (ELDA) in mice. The CSC-CAF crosstalk responsible for CSC induction was found to be mediated by Wnt5a signaling. CRISPR knockdown of Wnt5a in CAFs or treatment with a specific Wnt5a inhibitor abrogated the induction of CSCs by CAFs. Only cancer cells with ROR2, a Wnt coreceptor, respond to Wnt5a signaling triggered by CAFs and developed into CSCs. Responders were found to signal through a non-canonical Wnt pathway involving the coreceptor ROR2, protein kinase C (PKC), and cAMP Responsive Element Binding Protein 1 (CREB1). Inhibition of each of them prevented CSC induction and functional rescue experiments were performed to confirm the sequence of the Wnt5a-ROR2-PKC-CREB1 axis. Treatment of mouse xenografts, established by co-injection of CAFs and ovarian cancer cells, with the Wnt5a inhibitor sensitized them to carboplatin, and eliminated the CSCs in the residual tumors. Our results indicate that CAF-derived Wnt5a is instrumental in ovarian cancer CSC growth and maintenance. Targeting Wnt5a in tumor effectively prevents tumor relapse after cytotoxic chemotherapy by destroying suitable CSC-enriching microenvironment. In the long term, our studies will broaden the understanding of the mechanism of CSC maintenance by the tumor microenvironment and contribute towards the development of novel therapeutic approaches to prevent ovarian cancer chemoresistance and relapse.

***Basic Science*** ***Graduate Student***

# POSTER #19

## THE ROLE OF NOTCH ACTIVATION IN CANCER ASSOCIATED FIBROBLASTS IN PROMOTING OVARIAN CANCER METASTASIS

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Metastasis is the least understood aspect of cancer and accounts for ~90% of cancer-related deaths. Ovarian cancer (OC) is the most lethal gynecologic malignancy with most patients presenting with disseminated disease at diagnosis. Among all OC subtypes, high grade serous ovarian carcinoma (HGSOC) is the most prevalent and deadliest due to its extensive metastatic capability. The stromal cells of the tumor microenvironment (TME) form a supportive niche for the establishment of metastases via cross-talk with the invading OC cells. Studies have indicated that cancer associated fibroblasts (CAFs) are the predominant stromal component (~30-60%) and play an important role in HGSOC metastasis. CAFs aid in metastasis via paracrine secretion of growth factors and cytokines, ECM remodelling, immunosuppression, and induction of EMT in cancer cells. However, the role of OC-CAF mediated juxtacrine signaling pathway, remains poorly understood in HGSOC metastasis. By analyzing sc-RNA-seq datasets of HGSOC patient metastases, we have identified significant upregulation of the Notch signaling pathway in subpopulations of CAFs. The Notch pathway has largely been studied in cancer cells, while its role remains poorly understood in the context of CAFs and OC-CAF cross-talk. Treatment of OC patient tumor-derived CAFs with gamma-secretase inhibitor (GSI) *in vitro*, decreased the collagen-contraction ability of CAFs, indicating the role of Notch activation in CAF function. Importantly, CAFs cocultured with OC cells displayed significant induction of Notch downstream targets, Hes1 and Hey1 in comparison to monocultured CAFs. Treatment with epigenetic enzyme inhibitors significantly induced Hes1 and Hey1 in CAFs, indicating the role of chromatin remodeling by the OC-CAF cross-talk inducing Notch downstream signaling. We aim to further delineate the underlying mechanism using a heterotypic coculture model of OC cells and CAFs. The knowledge gained would help us design precise therapeutics targeting the CAFs to ‘normalize’ the TME and also combine them with existing tumor-targeting therapies, for better survivability of HGSOC patients.

***Basic Science*** ***Graduate Student***

# POSTER #20

## EXTRACELLULAR DEK ACTIVATES ANTIOXIDANT TARGETS AND DRIVES EXPANSION OF FUNCTIONAL, ROS-LOW HUMAN HEMATOPOIETIC STEM CELLS

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Umbilical cord blood (CB) is a promising source of hematopoietic stem cells (HSCs) for hematopoietic cell transplantation (HCT), a life-saving treatment, due to lower incidence of graft versus host disease and its availability. CB HCT is limited by fixed numbers of HSCs per unit of cord blood, thus there is a strong need to increase numbers of engraftable HSCs, for instance by expanding functionally competent HSCs *ex vivo*. We have shown that extracellular DEK protein, an epigenetic regulator that is secreted in response to stress and inflammation, is a novel hematopoietic regulatory chemokine. Treatment with extracellular DEK in growth stimulating media significantly enhances the expansion of engraftable HSCs in mouse bone marrow (BM) and human CB (Capitano, et al, *JCI,* 2019).

To examine mechanisms driving enhanced expansion of functional HSCs after DEK treatment, we performed RNA-sequencing on CB HSCs, multipotent progenitor cells (MPPs), common myeloid progenitors (CMPs), and granulocyte-macrophage progenitors (GMPs) after overnight DEK treatment in expansion media. All cell populations upregulated gene programs related to antioxidant responses upon DEK treatment. Overnight DEK treatment significantly reduced mean fluorescence of the activated reactive oxygen species (ROS) indicator CM-H2DCFDA in HSCs, MPPs, CMPs, and GMPs. Thus, DEK may induce antioxidant activity. We further examined whether DEK treatment affects mitochondrial metabolism, a source of ROS and tightly regulated process in hematopoiesis. Seahorse XFe analysis, which measures metabolic flux, showed DEK treatment of MLL-AF9 cells, a hematopoetic derived mouse AML cell line, significantly reduced mitochondrial oxygen consumption rate, suggesting a reduced reliance on oxidative phosphorylation as an energy source in DEK treated cells.

These data suggest that DEK treatment during expansion of primitive hematopoietic cells drives upregulation of antioxidant response genes, reduction of intracellular ROS levels, and may reduce the mitochondrial metabolic rate. These effects in a sense "mimic" the *in vivo* niche of HSCs, where they reside in low oxygen tensions, have low intracellular ROS, and exhibit low levels of oxidative phosphorylation. Thus, extracellular DEK treatment leads to expansion of functionally competent HSCs and reduction of oxidative stress. DEK may present a novel way to mimic physioxic conditions for enhancement of functional HSC expansion, and the elucidated mechanisms may be exploited for more efficient targeting of molecular programs critical for functional HSC competency, potentially providing new ways to enhance HCT.

***Basic Science*** ***Graduate Student***

# POSTER #21

## INDUCED ENDOREPLICATION BY AURORA B KINASE INHIBITION AS A MODEL FOR TUMOR HETEROGENEITY

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Endoreplication is an alternative cell cycle wherein cells undergo alternating G and S phases without dividing. While endoreplication can occur naturally to generate polyploid cells in growth and development, it can also be co-opted by cancer cells, leading to genome instability, tumor evolution, and cancer relapse; however, the mechanisms leading to these outcomes are unknown. We developed a cell culture method to generate induced endoreplicating cells (iECs) from diploid RPE-1 cells by treating cells with cell cycle inhibitors to study how changes in cell cycle dynamics lead to genomic heterogeneity and how cells with increased ploidy return to division. We found that Aurora B kinase inhibition resulted in a subtype of endoreplication called endomitosis, wherein cells enter mitosis but do not divide. Cells treated with a high concentration of Aurora B inhibitor failed at the metaphase/anaphase transition, resulting in daughter cells with multi-lobed nuclei. In contrast, cells treated with a lower concentration of Aurora B inhibitor failed at cytokinesis and resulted in binucleate cells. We found that cells with multi-lobed nuclei preferentially undergo DNA synthesis, suggesting that nuclear phenotype and/or the level of Aurora B inhibition may play a critical role in determining whether a cell can bypass the tetraploid checkpoint to become polyploid. To understand the molecular pathways contributing to endoreplication, we performed RNA-seq on iECs and found that there was an increase in the senescence-associated secretory phenotype signature and core senescence genes. Using fluorescence microscopy, we found that some, but not all, cells were positive for senescence markers, suggesting the response to Aurora B inhibition is heterogeneous. To ask whether polyploid iECs can resume proliferation, iECs were flow-sorted based on DNA content and plated in the absence of inhibitor. After an initial lag phase, a subset of cells began to rapidly proliferate and form colonies of mitotically dividing cells. We are currently assessing why only some cells return to division, and if senescence is a determinant factor. We are also re-expressing genes downregulated in iECs to ask if we can establish a gene re-expression network that stimulates return to division. Our studies provide a potential model for tumor heterogeneity that will help define molecular mechanisms that lead to endoreplication and promote return to division, and may provide new insights into how cancer cells evade normal cell cycle control.

***Basic Science*** ***Graduate Student***

# POSTER #22

## SUPPRESSION OF PP2A-B56A DRIVES EMT IN EGFR MUTANT NSCLC

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Lung cancer is the leading cause of cancer-related deaths worldwide. There are 2 main types of lung cancer: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Of these, NSCLC is the most common sub-type and accounts for 85% of lung cancer cases. In NSCLC, two of the most common driving alterations include EGFR (27%) and KRAS (32%), which are mutually exclusive mutations. Although KRAS has remained difficult to therapeutically target, many inhibitors have been developed and FDA-approved to target EGFR. While these therapeutics initially elicit a response, patients typically develop acquired resistance in less than a year, demonstrating a need for understanding these mechanisms that lead to targeted therapeutic resistance. One mechanism of acquired resistance that has been shown to occur is through a process known as epithelial-to-mesenchymal transition (EMT).

EGFR signals downstream to many effectors that promote cell survival and proliferation, and these factors can be negatively regulated by the serine/threonine phosphatase, Protein Phosphatase 2A (PP2A). PP2A consists of an “A” scaffolding subunit, a “C” catalytic subunit, and a “B” regulatory subunit, with the B subunit conferring substrate specificity. Based on the combination of these isoforms, there are over 90 potential complexes. Historically, PP2A has been studied as a single entity and has been identified as a tumor suppressor. Unlike many other tumor suppressors, PP2A is infrequently mutated in cancer, but is instead suppressed. One of the subunits, B56a, has shown tumor suppressive capabilities through suppression of the subunit leading to increased oncogenic phenotypes *in vitro*.

We find that low expression of B56a correlates with poor prognosis in NSCLC. Knockdown of PP2A-B56a through shRNA leads to increased vimentin and decreased e-cadherin by both mRNA and protein, a known signature of EMT. This suppression also leads to morphological changes consistent with an EMT such as increased migration. Additionally, these EMT phenotypes can be rescued in response to PP2A-B56a overexpression. Together these results implicate PP2A as a key factor in possible resistance mechanisms through EMT in EGFR mutant NSCLC.

***Basic Science*** ***Graduate Student***

# POSTER #23

## ADIPOSE TISSUE LOSS PRECEDES SKELETAL MUSCLE LOSS IN MURINE MODELS OF PDAC CACHEXIA

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Cachexia is the involuntary wasting of adipose and muscle tissue. Cachexia occurs in over 80% of patients with pancreatic adenocarcinoma cancer (PDAC), resulting in reduced survival and quality of life. We have previously shown that fat loss precedes lean tissue loss in PDAC cachexia. Furthermore, in our *in vitro* model, KPC conditioned media is sufficient to induce adipocyte lipolysis thereby inducing myotube wasting. These data suggest that changes to the adipose tissue microenvironment precede muscle wasting; however, this has yet to be determined. Therefore, we set out to characterize the adipose tissue microenvironment in early and late PDAC cachexia. We hypothesize that the adipose tissue microenvironment is disrupted early in PDAC cachexia. Male C57BL/6J (N=30) mice underwent orthotopic injection of $5x10^4$ KPC cells or SHAM surgery. Tissues were harvested every 3 days starting on day 6 post injection:6, 9, 12, 15, and 18. Tumors were palpable on day 9, and significantly increased in size from days 12 (0.612 ± 0.092g) to 18 (1.79 ± 0.309g). Fat mass, by echo MRI, pre-occurred as early as day 9, while there were no changes in lean mass till day 18. Epididymal and inguinal white adipose depots were reduced at days 15 and 18 in KPC compared to SHAM. Interestingly, skeletal muscle mass was also decreased at days 15 and 18 in KPC compared to SHAM. Next, using single nuclei RNA sequencing, we will identify if the cell types in adipose tissue change during cachexia development.

***Basic Science*** ***Graduate Student***

# POSTER #24

## ASSESSING HOW THE KINESIN-14 TAIL DOMAIN CONTRIBUTES TO CENTROSOME CLUSTERING

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Centrosome amplification is a hallmark of cancer that correlates with poor outcomes. Cells with centrosome amplification form multipolar spindles, which lead to multipolar cell divisions and lethal levels of aneuploidy. However, many cancer cells can cluster centrosomes and form bipolar spindles, which enhances cancer cell survival. Kinesin-14 proteins are important for centrosome clustering and are often amplified in many tumors. Inhibition of Kinesin-14 proteins in cancer cells with centrosome amplification leads to cell death; however, Kinesin-14s are thought to not be essential in normal cells. Therefore, targeting Kinesin-14s could provide a novel mechanism to selectively kill cancer cells. Kinesin-14s are minus end directed molecular motors that cross-link and slide both parallel and anti-parallel microtubules. Previous work from our lab showed that Kinesin-14s can cross-link microtubules using their ATP-dependent kinesin-like motor domain and a second microtubule domain in the tail domain. How a single microtubule binding domain in the tail could be involved in cross-linking of microtubules of opposite polarity is not known. To address this question, we mapped the regions of the tail that are important for microtubule binding and found two independent microtubule binding domains, which we named MBD1 and MBD2. Biochemical analysis of these domains supports the idea that MBD1 mediates anti-parallel microtubule cross-linking and MBD2 mediates parallel microtubule cross-linking. We propose that anti-parallel microtubule cross-linking through MBD1 would be critical for centrosome clustering. To test this idea in cells, we have done knockdown/rescue experiments using wild-type and mutant Kinesin-14s to ask the function of both MBD1 and MBD2, and if mutation of MBD1 impacts centrosome clustering. We have found that MBD2 is necessary for spindle length maintenance and that both MBD1 and MBD2 are needed for proper spindle localization. Preliminary data has implicated MBD1 in clustering induced multipolar spindles. We postulate that loss of MBD1 will reduce anti-parallel microtubule cross-linking between supernumerary centrosomes, leading to multipolar divisions and cancer cell death, and that targeting this domain as a therapeutic will not harm normal cells due to previously shown non-essential roles in cells that do not have centrosome amplification. Understanding the mechanisms by which Kinesin-14s cluster centrosomes will enable us to develop novel inhibitors that have the potential to treat cancers with centrosome amplification.

***Basic Science*** ***Graduate Student***

# POSTER #25

## ROLE OF THE INTRINSICALLY DISORDERED RNA BINDING PROTEIN EWS AS A ONCOGENIC TRANSCRIPTIONAL CO-REGULATOR

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The ETS family of transcription factors become aberrantly activated in multiple cancers. This activation is dependent on the co-activator EWS in both prostate cancer and Ewing sarcoma. EWS is an intrinsically disordered RNA binding protein (ID-RBP). ID regions have no set secondary structure which allows for dynamic and transient interactions with other molecules. The ID region of EWS promotes self-binding, forming phase separated granules, and acts as a transcriptional activation domain. Mutation of the ID region of ID-RBP proteins prevents phase separation, activation, and tumorigenesis. This implies that phase separation is linked with activation and important for tumorigenesis. However, how phase separation may contribute to EWS activation and tumorigenesis is not yet known. The objective of this study is to investigate the mechanism and necessity of phase separation in EWS activated cancers. To determine the necessity of phase separation in oncogenesis I will create artificial EWS/FLI1 fusions where EWS is replaced by domains that are exclusively able to phase separate or transactivate. I will examine the oncogenic ability of these artificial fusions through phenotypic and functional assays including clonogenic survival and soft agar assays. Because EWS is primarily found in phase separated granules in cells, there is the potential that a membraneless organelle has a role in EWS activation. In particular, we are interested in the paraspeckle, a nuclear membraneless organelle involved in transcriptional regulation and is known to contain EWS. The role of the paraspeckle in EWS activation will be determined by knocking down the paraspeckle and examining the effect on oncogenic phenotypes and gene expression. This research will further our understanding of the importance of phase separation in tumorigenesis and the role of the paraspeckle in cancer.

***Basic Science*** ***Graduate Student***

# POSTER #26

## STAT3 AND LSD1 PROMOTE ENTEROENDOCRINE CELL FATE TO IMPACT COLON INFLAMMATION

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Inflammatory bowel disease (IBD) affects millions of people in the US, with case frequency on the rise. Characterized by chronic inflammation of the gastrointestinal tract, IBD is very debilitating and puts people at higher risk of developing colorectal cancer. Enteroendocrine cells (EECs) are specialized secretory cells within the colon epithelium that secrete hormones to regulate absorption of water and nutrients from the lumen of the colon. Recent studies have implicated EECs as key regulators of mucosal immunity through the sensing of microbes and the secretion of cytokines and chemokines, suggesting that EECs have an import role in colon inflammation. Additionally, EECs may have clinical relevance to IBD as EECs have been observed to be increased in IBD patients and positively correlated with markers of inflammation. Despite these observations however, the mechanism leading to the increase of EECs in IBD and the role of EECs in inflammation is not well understood. The Signal Transducer and Activator of Transcription 3 (STAT3) is a transcription factor typically studied for its role in immunity, including in IBD. Additionally, STAT3 has been shown to regulate cell differentiation, including in spermatogonia and pancreatic beta cells. Uniquely, in both cases STAT3's role in cell differentiation centered around increasing the expression of *NEUROG3*, a transcription factor that is essential for the differentiation of EECs in the colon. Lysine demethylase 1 (LSD1) is a chromatin modifying enzyme that is found in numerous transcriptional activator and repressor complexes. Included among those complexes is the CoREST complex, which includes LSD1, HDAC1/2, and one of three CoREST proteins (CoREST 1/2/3). Previously, we have implicated LSD1 in the differentiation of EECs in colon cancer and interestingly, LSD1 has been shown to demethylate STAT3 to enhance STAT3's transcriptional activity in response to IL-6 stimulation. Here, using organotypic human colon organoids and HT29 colon cancer cells, both of which contain all the major cell lineages found in the colon, we show that STAT3 promotes EEC differentiation in a calcium signaling dependent manner. Furthermore, we show that LSD1 demethylates STAT3 to promote EEC differentiation in a CoREST2 dependent manner. Finally, using bacterial extracellular protein lipopolysaccharide as an inflammatory stimulus, we demonstrate that EECs play an important role in the transcriptional inflammatory response in the colon. Future directions will be to assess the importance of the methylation of status of STAT3 in EEC differentiation, determine where in the genome STAT3 is binding to promote EEC differentiation, and evaluate the role of EECs to attract immune cells and promote inflammation. Non-responsiveness to treatment and relapse are common in IBD, thus decreasing EECs through targeting STAT3 and LSD1 may have therapeutic benefits to IBD patients.

***Basic Science*** ***Graduate Student***

# POSTER #27

## DISCOVERY OF A DESTABILIZING LIGAND OF PROTEASOME UBIQUITIN RECEPTOR RPN-13, A THERAPEUTIC TARGET FOR HEMATOLOGICAL CANCERS

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The proteasome, a large multi-catalytic complex of ~2.5 MDa, serves as the major protein degradation pathway in eukaryotic cells. The 20S core particle (20S CP) cleaves proteins into short peptide fragments, and when complexed with the 19S regulatory particle (19S RP), the newly synthesized 26S proteasome is capable of degrading proteins that have been tagged with ubiquitin. This has made it a desirable target for treatment of hematological cancers due to their dependency on high 26S proteasomal activity. Targeting the 19S RP rather than the 20S CP with covalent inhibitors has shown promise in hematological cancer treatment. Rpn-13, a ubiquitin receptor of the 19S RP, is a therapeutic target of interest as it is non-essential in healthy cells but is important for the survival of blood cancers such as multiple myeloma (MM). Our goal is to develop non-covalent scaffolds that bind to the Pru (Pleckstrin-like receptor for ubiquitin) domain of Rpn-13. This work was accomplished in collaboration with Atomwise, a biotechnology company that uses artificial intelligence for drug discovery. Based on their computational analysis, we tested a library of compounds through various biophysical and chemical biology studies such as biochemical & cellular thermal shift, fluorescence polarization, and cell viability assays, in addition to structural biology analysis by 2D protein NMR. From the screen, we have validated a new binder, TCL-1, of the Pru domain on Rpn-13. TCL-1 has demonstrated selectivity for Rpn-13 by eliciting mild toxicity in hematological cancer cells (Ramos & MM.1R), and no toxicity to non-malignant cells (HEK-293T) up to 100μM. TCL-1 also demonstrated dose-dependent destabilization of Pru biochemically. Confirmation of the binding site by 2D NMR will allow us to further optimize TCL-1's scaffold through structure-based drug design, with the aim of developing more potent and selective non-covalent Rpn-13 inhibitors.

***Basic Science*** ***Graduate Student***

# POSTER #28

## CHARACTERIZATION OF ZNF423 IN NF1-RELATED, PRC2-DEFICIENT MALIGNANT PERIPHERAL NERVE SHEATH TUMORS

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Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant disorders with a predisposition to cancer, caused by inactivating mutations in the tumor suppressor gene *NF1*. The leading cause of death in NF1 patients is the development of malignant peripheral nerve sheath tumors (MPNST), which typically emerge from the progression of benign plexiform neurofibromas (PNF) to pre-cancerous atypical neurofibromas (ANF) and is characterized by loss of the *CDKN2A/B* tumor suppressor loci. In addition to the loss of *NF1* and *CDKN2A/B*, there is a high frequency of alterations in either *EED* or *SUZ12*, which encode components of the polycomb repressive complex 2 (PRC2), a chromatin regulator that maintains gene silencing. To investigate the effect of PRC2 loss on MPNST gene expression, we restored *SUZ12* in two PRC2-deficient MPNST lines and performed RNA-sequencing, identifying fourteen common transcription factors downregulated by PRC2 reconstitution. Amongst those downregulated was ZNF423, a transcription factor expressed in numerous immature cell populations—including neural precursors that form mature olfactory neurons. Interestingly, in murine-cells-of-origin where both *Nf1* and *Cdkn2a (Arf)* were inactivated, ZNF423 was significantly de-repressed compared to controls. Our preliminary experiments using shRNA targeting ZNF423 in human MPNST lines indicated that ZNF423 reduction significantly reduced their viability and proliferation. Ongoing studies will further delineate the role of ZNF423 in human and murine MPNST models using omics approaches, cell-based phenotypic assays, and *in vivo* studies.

***Basic Science*** ***Graduate Student***

# POSTER #29

## A COMPLEX SIGNATURE NETWORK THAT CONTROLS THE UPREGULATION OF PRMT5 IN COLORECTAL CANCER

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Protein arginine methyltransferase 5 (PRMT5) deregulation has emerged as an important prognostic indicator in human cancers. Through aberrant methylation-mediated epigenetic modification of signaling molecules, PRMT5 overexpression contributes to the dysregulation of a variety of cellular processes related to cancer progression. However, the mechanisms governing PRMT5 expression levels in cancer remain largely unknown. In this study, we examined factors that regulate PRMT5 expression at multiple levels. We mapped three regions of the proximal promoter of PRMT5 and identified NF-Ya, SMAD3, and ZNF143 as part of a key signature network node regulating PRMT5 expression in HT29 colorectal cancer (CRC) cells. Importantly, we provide evidence that knockdown or ligand-induced activation of SMAD3, and ZNF143 led to changes in PRMT5 transcript and protein levels, respectively. We showed that PRMT5 expression positively correlates with both TGF-β2 and ZNF143 expression, suggesting that activation and/or upregulation of these proteins may be partly responsible for PRMT5 overexpression in a subset of CRC patients. Collectively, our data present a complex model that involves cell-autonomous induction of PRMT5 in CRC cells by transcriptional mechanisms and upregulation of PRMT5 mRNA and protein, which encompasses processes involving gene amplification and increased transcription and protein turnover rates.

***Basic Science*** ***Graduate Student***

# POSTER #30

## ARF6 INHIBITION ENHANCES T CELL-MEDIATED CYTOTOXICITY IN TRIPLE NEGATIVE BREAST CANCER

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Triple negative breast cancer (TNBC) has the poorest clinical outcome owing to the lack of effective treatments out of all the breast cancer subtypes. Immune checkpoint blockade (ICB) therapies have shown promise in these patients but only a small proportion of patients respond to ICB therapy partly due to immune evasion. A primary mechanism by which tumor cells evade immune surveillance is downregulation of antigen presentation by decreasing the levels of antigen-MHC-I (major histocompatibility complex-I) complexes on the cancer cell surface. However, there are no known targets that would boost antigen presentation in the tumor cells and increase T cell functionality simultaneously to increase the efficacy of ICB. The small GTPase ARF6 has been implicated in the internalization of MHC-I molecules into the cell. Additionally, ARF6 also plays a role in $CD8^+$ T cell activation by reorganization of the actin cytoskeleton and inhibits the formation of the immunological sypnase. We hypothesize that by inhibiting ARF6 in both tumor cells and $CD8^+$ T cells, T cell-mediated cytotoxicity would be increased. In cancer cells, ARF6 inhibition will inhibit endocytosis of MHC-I complexes and enhance antigen presentation, while in $CD8^+$ T cells, Arf6 inhibition will promote the formation of the immunological synapse. We tested the dose-dependent effect of an ARF6 inhibitor NAV-2729 on the GTPase activity of ARF6 in tumor cells as it specifically inhibits ARF6 by blocking its GTP-binding domain. We observed a dose-dependent decrease in ARF6-GTP levels with NAV-2729 treatment. To this end, we inhibited ARF6 via the small molecule inhibitor NAV-2729 or knockdown by shRNA in murine and human TNBC cells and found that antigen presentation is enhanced when MHC-I mean fluorescence intensity (MFI) was measured by flow cytometry. We also inhibited ARF6 in $CD8^+$ T cells by NAV-2729 pretreatment, activated them *in vitro* and measured their functionality by flow cytometry. Interestingly, inhibitor treatment led to higher IFNg, TNFa and IL2 production. In a T cell cytotoxicity assay, we observed increased T cell cytotoxicity in the coculture of T cells and cancer cells when either the tumor cells or $CD8^+$ T cells were treated with inhibitor. Collectively, we show here that ARF6 inhibition in tumor cells and $CD8^+$ T cells will enhance tumor cell killing due to enhanced antigen presentation and T cell functionality respectively. These results give us more insight into the use of Arf6 inhibitors in combination with ICB, thus enhancing treatment efficacy.

***Basic Science*** ***Graduate Student***

# POSTER #31

## LONG NONCODING RNAS ARE ABUNDANT TARGETS OF ADAR3 IN GLIOBLASTOMA

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Adenosine-to-Inosine (A-to-I) editing occurs in almost 60% of human transcripts and is catalyzed by Adenosine Deaminases Acting on dsRNA (ADAR) family of RNA-binding proteins. In humans, A-to-I editing is primarily mediated by two ubiquitously expressed deaminases, ADAR1 and ADAR2. Both ADAR1 and ADAR2 are essential for normal neurological function and are associated with several pathologies including cancer, Alzheimer's, epilepsy and Aicardi-Goutières syndrome (AGS). Hence, extensive research has been directed towards understanding gene regulatory functions of the active deaminases. On the contrary, despite being discovered two decades ago, the physiological role of the brain-specific, deaminase-deficient ADAR family member, ADAR3, is largely unknown.

Loss of ADAR3 leads to altered gene expression profiles and is associated with learning and memory deficiencies in mice. Furthermore, studies from our lab have revealed that

glioblastoma tissue samples have elevated ADAR3 expression when compared to adjacent normal tissues, and increased ADAR3 expression promotes temozolomide resistance of glioblastoma cells due to aberrant activation of NF-kB. Literature also demonstrates that ADAR3 is capable of binding both single and double stranded RNA molecules. However, molecular targets of ADAR3 and the mechanism underlying how ADAR3 binding to target transcripts influence oncogenic gene expression profiles are still unknown. Our central hypothesis is that ADAR3 regulates glioblastoma progression through directly binding to target transcripts and modulating the tumor transcriptome and/or proteome by regulating transcript localization. In this regard, a high-throughput approach was employed in U373 glioblastoma cells to identify transcripts bound by endogenous ADAR3. We found that ADAR3 binds 1435 RNA targets of which almost 30% are long non-coding RNAs (lncRNAs). Since lncRNAs are known to play an important role in gene expression regulation in various cancers, our current efforts are directed towards delineating the global mechanism by which ADAR3 regulates localization and expression of bound lncRNAs in the context of glioblastoma progression. Concurrently, we are also trying to understand if binding to lncRNAs is a function unique to ADAR3 or extends to other ADAR family members since prior to this, transcriptome-wide studies of ADARs haven't identified lncRNAs as major targets. As very little is known about the molecular and cellular functions of ADAR3, insights gained from this study will be highly valuable in shedding light on the oncogenic potential of ADAR3.

***Basic Science*** ***Graduate Student***

# POSTER #32

## NOVEL ROLE FOR PP2A IN REGULATING EGFR SIGNALING IN KRAS MUTANT PDAC

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Pancreatic ductal adenocarcinoma (PDAC) stands to become the 2nd most deadly cancer by 2030. Over 90% of PDAC patients have driver mutations in the small GTPase, KRAS. The most common KRAS mutation in PDAC, $KRAS^{G12D}$, has yet to be successfully therapeutically targeted, thus there is a critical need for alternative therapeutic targets to improve patients outcomes. Protein phosphatases are master regulators of cellular signaling cascades. Protein phosphatase 2A (PP2A) is a large family of Ser/Thr phosphatases which negatively regulate many downstream targets of KRAS. Small molecule activators of PP2A have emerged as promising anti-cancer agents. Studies have shown that the pharmacological activation of PP2A suppresses oncogenic PDAC signaling pathways, however this response is heterogeneous, suggesting that PP2A may have unique functions in PDAC. Historically, studies have approached PP2A as one entity despite the fact that PP2A encompasses over 90 distinct complexes. However, as the individual roles of specific subunits are interrogated, tissue and context specific roles for PP2A have now emerged. The subunit B56α has been previously implicated as a tumor suppressor but has remained understudied in PDAC.

Using pharmacological activation of PP2A, as well as overexpression and knockdown studies, we have identified a novel role for PP2A-B56α in epidermal growth factor receptor (EGFR) signaling in PDAC. EGFR signaling is a significant signaling node in PDAC: EGFR expression is increased during PDAC progression and loss of EGFR prevents mutant KRAS-driven pancreatic tumorigenesis. Activation of PP2A-B56α leads to the suppression of specific oncogenic pathways, a subset of PDAC cell lines displaying increased secretion of EGFR ligands through the sheddase ADAM17, activation of EGFR, and downstream oncogenic phenotypes.

***Basic Science*** ***Graduate Student***

# POSTER #33

## THE TARGET GENE REGULATORY NETWORK OF MIR-497 IN ANGIOSARCOMA

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Angiosarcoma (AS) is an aggressive, highly metastatic vascular cancer with a 5-year survival rate of only 30 percent. Due to the rarity of AS, treatment options for patients are limited, therefore more research is needed to identify possible therapeutic vulnerabilities. Our lab previously found that endothelial-specific deletion of *Dicer1* drives AS development in mice. Given the essential role of DICER1 in canonical microRNA (miRNA) biogenesis, this finding suggests that miRNAs may be important in AS development, therefore warranting further research into their role. MiRNAs have been implicated in several other cancers, however, their role in AS has not been studied extensively. After testing several miRNAs previously suggested to have a tumor-suppressive role in AS, microRNA-497-5p (miR-497) suppressed cell viability most significantly in AS cell lines. In other cancers, miR-497 is generally reported to be downregulated in malignant tissue compared to normal and has been suggested to have a tumor and metastasis-suppressing role. In addition to the observed effects on cell viability, we also observed that miR-497 expression leads to increased apoptosis and inhibited cell migration in AS cells *in vitro*. Additionally, miR-497 expression suppressed tumor formation and metastasis in a subcutaneous murine AS model. To better understand the mechanism of how miRNAs elicit phenotypes, it is pertinent to identify possible clinically relevant target genes. Using a combination of RNA-sequencing data in an AS cell line, expression data from AS patient tumors, and target prediction algorithms, we identified four clinically relevant miR-497 target genes for further study, and have validated that miR-497 directly regulates these target genes. Additionally, we have found that these gene targets may impact cell viability and cell migration through preliminary shRNA-mediated knockdown and pharmacological inhibition experiments. This work will give insight into the role of miR-497 and its target genes in AS pathogenesis. The knowledge gained may be useful for future efforts to develop therapeutics for AS.

***Basic Science*** ***Graduate Student***

# POSTER #34

## THERMAL PROTEOME PROFILING OF TRIPLE NEGATIVE BREAST CANCER CELLS FOLLOWING DNA DAMAGE INDUCTION THROUGH COMBINATION TREATMENT WITH IB-DNQ AND RUCAPARIB

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Triple negative breast cancer (TNBC) accounts for 15-20% of all breast cancer cases, and patients have a higher risk of relapse and decreased survival. TNBC lacks the estrogen, progesterone, and epidermal growth factor 2 receptors; consequently, commonly used hormonal therapies are ineffective. One potential TNBC-specific target is NAD(P)H:quinone oxidoreductase 1 (NQO1), which is often highly expressed in TNBC tissue. The NQO1 bio-activatable drug isobutyl-deoxynyboquinone (IB-DNQ) acts as a futile cycling substrate for NQO1 that leads to an accumulation of reactive oxygen species and subsequent DNA damage. Upon this damage, PARP1 is hyperactivated to initiate DNA repair mechanisms. PARP1 inhibition (via Rucaparib) combined with IB-DNQ should increase DNA damage to synergistically induce cell death specifically in cells highly expressing NQO1.

To further understand the mechanism of Rucaparib and IB-DNQ-induced cell death, MDA-MB-231 TNBC cells expressing endogenous NQO1 or the rapidly degraded NQO1*2 variant were treated with lethal and sublethal doses of IB-DNQ, Rucaparib, or a combination treatment. Samples were analyzed using global proteomics, phosphoproteomics, and thermal proteome profiling (TPP). Kinase substrate enrichment analysis of phosphoproteomics data identified changes in kinase activity. Following TPP, the R-based analysis workflow Inflect-SSP generated melt curves using the protein abundance values from each temperature point and calculated z-scores to assign p-values to shifts in melt temperature. Inflect-SSP also extends to STRING and Panther to identify any enrichments in known protein-protein interactions and cellular pathways for significantly changed proteins.

We hypothesized that in the presence of NQO1, IB-DNQ and Rucaparib treatment would result in changes in phosphorylation and protein thermal stability, illuminating how the drugs alter the TNBC proteome. IB-DNQ and Rucaparib combination treatment of TNBC cells triggered large changes in the phosphoproteome while low dose IB-DNQ treatment alone did not induce extensive changes in phosphorylation networks. Histone H2AX was among the several proteins identified to have increased phosphorylation when cells were treated with the combination of IB-DNQ and Rucaparib. This finding validates that the drugs induced persistent DNA damage as phosphorylated H2AX at Serine 139 is a marker of DNA damage. We also observed a decreased melt temperature for H2AX following combination treatment suggesting that increased phosphorylation of H2AX may destabilize the protein. Thermal proteome profiling identified significant protein stabilization within the cell cycle kinase network when cells were treated with the sublethal dose of IB-DNQ. Together, these results indicate that IB-DNQ and Rucaparib impact cell cycle progression. The data also suggests that TPP has increased sensitivity for kinase network changes compared to phosphoproteomic analysis which demonstrates that TPP has greater sensitivity to detect drug-induced changes in TNBC cell cycle.Our findings illustrate that paired TPP-phosphoproteomics datasets reveal complementary insights into cell signaling following drug treatment.

***Basic Science*** ***Graduate Student***

# POSTER #35

## GCN2 INHIBITION SENSITIZES PROSTATE CANCER CELLS TO LOSS OF P53/P21 CELL CYCLE CONTROL

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The Integrated Stress Response (ISR) is central for cells to adapt and survive during nutrient starvation. The ISR features a family of related protein kinases that phosphorylate eukaryotic initiation factor 2 (eIF2) to induce the translational and transcriptional expression of genes that function in amino acid synthesis and uptake, alleviation of oxidative stress, and control of the cell cycle and viability. We recently demonstrated that the activation of the eIF2 kinase GCN2 is critical for amino acid homeostasis and growth and progression of prostate cancer (PCa) in culture and in mouse models. GCN2 serves as a nutrient sensor in the ISR and is a critical regulator of transport of essential amino acids (EAAs). Genetic or pharmacological depletion of GCN2 results in lowered expression of amino acid transporters and severe depletion of intracellular EAAs in PCa. Although loss of GCN2 in PCa cells reduces proliferation, depletion of this eIF2 kinase leads to cell stasis and not cell death.

We hypothesize that the static phenotype induced by GCN2 inhibition in PCa cells will render these cells vulnerable to cell cycle modulation and is a potential therapeutic target. To address this idea, we utilized models of androgen-sensitive and castration-resistant PCa and normal non-tumorigenic prostate epithelial cells. We determined that GCN2 inhibition in PCa cell lines results in cell cycle arrest at the G1 phase of the cell cycle and is dependent on expression of the cell cycle inhibitor p21 by processes involving induction of the tumor suppressor p53. Of interest, induced p53/p21 and the attendant G1 arrest by GCN2 inhibition can be reversed by supplementation with EAAs, suggesting amino acid limitation is critical for the p53/p21-dependent checkpoint control of the cell cycle. Importantly, the combination of GCN2 inhibition and p53 or p21 knockdown resulted in PCa cell death and apoptosis. Our results suggest that inhibition of GCN2 in PCa cells causes severe starvation for EAAs, triggering a cell cycle checkpoint and G1 cell cycle arrest by a p53/p21-dependent mechanism, which is central for PCa survival. As a consequence, combination therapies targeting GCN2 and p53/p21 pathway are suggested to be a promising strategy for treatment of PCa and other cancers addicted to the ISR for alleviation of nutrient stresses.

***Basic Science*** ***Graduate Student***

# POSTER #36

## INTERFERENCE OF THE MISMATCH REPAIR PATHWAY WITH THE DNA REPLICATION PROCESS

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Maintenance of a stable genome requires high-fidelity DNA replication and repair. The cell ensures maximum accuracy of the duplication process by employing proof-reading polymerases, efficient DNA repair pathways, and cell cycle checkpoints. Of specific significance during this act of genome safeguarding is the mismatch repair (MMR) pathway since it immediately follows replication, correcting any errors made by the DNA polymerase. This protective pathway, however, has also been implicated in causing genomic instability, by contributing to trinucleotide repeat (TNR) expansions, a leading cause of several neurological disorders. Several studies have shown that the mismatch binding protein, Msh2-Msh3, interferes with replication and repair proteins to promote expansions through binding and stabilizing the secondary structures formed when TNR sequences, especially CAG/CTG repeats, are single-stranded. A well-known path to TNR expansion is through Msh2-Msh3 interference with the lagging strand replication proteins FEN1 and DNA Ligase I (LigI). We have also found that elevated levels of Msh2-Msh3 interfere with efficient replication, potentially through Okazaki fragment processing. Together, these observations suggest the possibility that Msh2-Msh3 also interferes directly with Pol δ, the lagging strand polymerase, to further promote genetic instability, including TNR expansions. Msh2-Msh3 has specificity for a variety of DNA structures, some with a greater affinity over others, which modulate Msh2-Msh3's ATP binding/hydrolysis activity and interactions with partner proteins. Using a variety of replication intermediate substrates, we observed substrate specific influence of Msh2-Msh3 on Pol δ activity. We were also interested in understanding the regulation of Msh2-Msh3 in the context of these DNA structures. Previous studies from our lab show that lysine acetylation significantly alters the performance of replication proteins such as Pol δ, RPA, and Rad27$^{FEN1}$. Furthermore, a recent study indicates that the acetylation status of Msh2-Msh3 affects the rate of TNR expansions. Here, we show some preliminary data on how the lysine acetylation of Msh2-Msh3 impacts its functioning. We anticipate that our results and continued research into Msh2-Msh3's contribution to genetic instability will shed light on the mechanisms of Msh2-Msh3's aberrant functioning and provide clues of underlying causes and possible solutions for the same.

***Basic Science*** ***Graduate Student***

# POSTER #37

## ASPARAGINE BIOAVAILABILITY REGULATES THE TRANSLATION OF MYC ONCOGENE

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Non-essential amino acids have been shown to have diverse functions in tumor in tumor growth and proliferation beyond serving as mere biosynthetic substrates. While previous investigations have predominantly focused on the most abundant amino acid glutamine, recent studies have highlighted the roles of other non-essential amino acids in the context of cancer and other pathological conditions. This makes nutrient restriction a compelling strategy to treat the disease.

Using lymphoid cancer cells as a model, we found that asparagine depletion acutely reduces the expression of c-MYC protein without changing its mRNA expression. Furthermore, asparagine depletion inhibits the translation of MYC mRNA without altering the rate of MYC protein degradation. Of interest, the inhibitory effect on MYC mRNA translation during asparagine depletion is not due to the activation of the general controlled nonderepressible 2 (GCN2) pathway and is not a consequence of the inhibition of global protein synthesis. In addition, both the 5' and 3' untranslated regions (UTRs) of MYC mRNA are not required for this inhibitory effect. Finally, using a MYC-driven mouse B cell lymphoma model, we found that shRNA inhibition of asparagine synthetase (ASNS) or pharmacological inhibition of asparagine production can significantly reduce the MYC protein expression and tumor growth.

MYC is a very potent oncogene and is frequently dysregulated in cancers. While MYC has been shown to regulate the expression of metabolic genes, our studies with asparagine restriction show a reciprocal interaction between the metabolic microenvironment and MYC expression. Our results also indicate a novel mechanism of translation inhibition independent of canonical regulators of translation initiation. Finally, our work also expands on the clinical application of the chemotherapeutic L-Asparaginase to refractory lymphomas by combining it with a suitable electron transport chain inhibitor.

***Basic Science*** ***Graduate Student***

# POSTER #38

## ZNF217 AND NRG1/ERBB3 SIGNALING PROMOTE ENDOCRINE THERAPY RESISTANCE VIA THE LIGAND-INDEPENDENT ACTIVATION OF ER ALPHA

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Around 70% of breast cancer tumors are estrogen receptor positive (ER+), while ~33% of these tumors eventually relapse as recurrent metastatic tumors resistant to endocrine therapies (tamoxifen, fulvestrant). We identified the oncogene and transcription factor ZNF217 (human)/Zfp217 (mouse) as an ER modulating protein that drives tamoxifen and fulvestrant resistance. To study the role of Zfp217 in tamoxifen response *in vivo*, we treated mice with PyMT ± Zfp217 tumors with tamoxifen. Vector-expressing control tumors significantly responded to tamoxifen, while tumors overexpressing Zfp217 did not. Treatment with fulvestrant gave similar results.

The objective of this study was to identify the molecular mechanism of how ZNF217 causes endocrine therapy resistance. Ligand independent activation of ER by growth factor signaling pathways is a main mechanism of endocrine therapy resistance. Significantly, ZNF217 directly activates the ErbB3/AKT signaling pathway. We investigated transcriptomic and genomic changes driven by ZNF217 & ErbB3 signaling activated by neuregulin (NRG1) and how they drive tamoxifen resistance. We identified a novel prognostic ZNF217- and NRG1 (ligand of ErbB3)-dependent gene expression signature by RNA-Seq and noncanonical ER binding sites in the genome by ChIP-Seq in MCF7 cells. We also discovered ER- and NRG1-dependent ZNF217 binding sites in the MCF7 cells using CUT&RUN sequencing. These transcriptomic and genomic changes correlated with lower survival in ER+ breast cancer patients receiving tamoxifen and identified key pathways altered in a ZNF217-dependent manner after NRG1 induction. After ChIP-Seq and CUT&RUN data integration, we identified both PCK1 and PHGDH as novel inducers of tamoxifen resistance that rewire cellular metabolism. In MCF7 cells, both genes induced endocrine therapy resistance, while their deficiency made cells more susceptible to endocrine therapy. Significantly, overexpression of Pck1 in murine mammary tumors accelerated tumor burden, decreased survival, and promoted endocrine therapy resistance.

In summary, NRG1 induces a differential ZNF217-dependent gene expression signature and noncanonical ER genomic binding in ER+ breast cancer cells. Significantly, two targets, PCK1 and PHGDH, promote endocrine therapy resistance *in vivo* and are themselves potential therapeutic targets.

***Basic Science*** ***Graduate Student***

# POSTER #39

## THE ROLE OF PP2A-B56A IN KRAS-MEDIATED PANCREATIC TUMORIGENESIS

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Protein phosphatase 2A (PP2A) is a major serine/threonine phosphatase that regulates many cellular pathways including KRAS, whose oncogenic mutation is prevalent in 95% of patients with Pancreatic Ductal Adenocarcinoma (PDAC). Previous research has identified a decrease in global PP2A activity, as well as an increase in the expression of PP2A inhibitors, in PDAC cell lines. These studies suggest that suppression of PP2A activity may be important in PDAC maintenance.

The active PP2A holoenzyme consists of 3 subunits: the scaffolding subunit (A), the catalytic subunit (C), and the regulatory subunit (B). There are 20+ different B subunits that can be incorporated into the PP2A holoenzyme. The diversity of B subunits is responsible for substrate specificity. While global PP2A has tumor suppressive capabilities, the regulation of specific pathways by PP2A can dramatically change based on PP2A holoenzyme composition. Specifically, the B56α subunit of the heterotrimeric PP2A holoenzyme has been shown to negatively regulate cellular transformation and has decreased expression in PDAC, indicating that B56α suppression may aid in PDAC tumorgenicity. Therefore, there is a critical need to understand the mechanisms that alter PP2A function and substrate targeting.

*Our research aims to investigate the impact of oncogenic KRAS on PP2A-B56α activity and how suppression of B56α impacts the initiation and progression of PDAC.* Our preliminary studies suggest that induction of $KRAS^{G12D}$ increases the expression of cancerous inhibitor of PP2A (CIP2A), indicating that PP2A suppression may be an early event in PDAC initiation. Consistent with this hypothesis, our *in vivo* data show that the loss of B56α in the context mutant KRAS accelerates PDAC initiation, increasing the formation of precursor lesions. Additionally, loss of B56α in *ex vivo* acinar-to-ductal metaplasia assays promotes cellular plasticity in a cell-autonomous manner, thereby accelerating KRAS-dependent transdifferentiation. Future studies will investigate how mutant KRAS expression effects overall PP2A phosphatase activity, PP2A holoenzyme formation, and sequestration of B56α by endogenous inhibitors to further understand how suppression of PP2A-B56α contributes to development of PDAC. Together, these studies identify PP2A as a critical regulator of KRAS-induced cellular plasticity and suggest that the therapeutic reactivation of PP2A may be a novel therapeutic strategy in PDAC patients.

***Basic Science*** ***Graduate Student***

# POSTER #40

## CHARACTERIZING THE MOLECULAR MECHANISM OF PH SENSITIVE IONIZABLE RESIDUE NETWORKS IN ONCOGENES

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Transient increases in the intracellular pH (pHi) of mammalian epithelial cells (7.0-7.6) drives normal cellular behaviors like cell-cycle progression and migration while increased pHi is a hallmark of cancer. Normal and pathological pH-sensitive cell behaviors are driven by pH sensors, or proteins that undergo large structural and functional changes with physiological changes in pHi. Most identified pH sensors rely on titratable histidine residues for pH-sensitive function, but amino acids such as Asp, Glu, and Lys can have their pKas shifted into the physiological range depending on protein environment. However, the potential role for these ionizable residues in regulating pH-dependent protein function is understudied. We predict that some pH sensors function through cooperative ionizable residue networks (such Glu, Asp, and Lys) that lack histidine but have a collective pKa in the physiological range (7.0-7.6). Using computational and biochemical approaches, we investigated the potential for this mechanism to underlie the pH sensitive functions of phosphatidylinositol-3-kinase (PI3K), Janus Kinase 2 (JAK2), and Tyrosine-protein phosphatase non-receptor type 11(SHP2). We identified networks of ionizable residues with upshifted pKas at the SH2 domain interface of these proteins that may play a role in pH sensing. Because SH2 domains are structurally conserved, this pH-sensing mechanism could be conserved in kinases and phosphatases with inhibitory SH2 domains. Better understanding of how ionizable networks respond to pH will transform our understanding of the molecular mechanisms of pH sensitive proteins that mediate pH sensitive normal and cancer cell responses.

***Basic Science*** ***Graduate Student***

# POSTER #41

## ANALYZING MOUSE PRIMARY MESOTHELIAL CELL PROTEOMICS TO IDENTIFY CANDIDATE PROTEINS CONTRIBUTING TO GENDER- AND AGE-ASSOCIATED DIFFERENCES IN COLORECTAL CANCER METASTATIC PERITONEAL RECEPTIVITY

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As the third most lethal type of cancer in the U.S, colorectal cancer (CRC) kills more than 52,000 patients per year, with most patients dying of metastatic diseases. The peritoneal cavity is one of the major metastatic sites for CRC (10-35%). Metastasis is initiated by adhesion of CRC cells to peritoneal mesothelial cells. Epidemiologic studies suggest that female colorectal cancer patients have better survival than males; however this gender preference in survival was only observed under age 65, suggesting an age-related component to CRC survival. The goal of this study is to investigate the effect of age and gender differences in CRC intra-peritoneal (i.p.) metastatic success. Initial experiments analyzed metastatic tumor burden in cohorts of varied age and gender.Red fluorescent protein (RFP)-tagged murine MC-38 colon adenocarcinoma cells ($10^5$) were injected i.p. into cohorts of Aged (20 mo., approximates a ~65 y.o. human) or Young (4 mo, approximates a ~25 y.o. human) female or male mice. Tumor burden was monitored by longitudinal live imaging of the RFP signal for 4 weeks. Overall aged male mice showed more tumor burden than the others, consistent with the epidemiologic data. Males have more CRC metastatic lesions on visceral adipose relative to young males and to females of both ages. To identify differentially expressed molecules that may lead to varied metastatic success in the cohorts, we isolated mouse primary mesothelial cells (MPMC) lining the peritoneal cavity of tumor naïve mice for proteomic analysis. MPMC lysates underwent the STrap protocol, and were subjected to bottom-up proteomic analysis in the Notre Dame Mass Spectrometry Core using an LC-electrospray-orbitrap-orbitrap mass spectrometry. Overall, we identified ~2000 proteins per group with ~100 differentially expressed proteins. Among these, we identified proteins including CD166, Prl1 & 2, and Clusterin, as potentially contributing to the different metastatic patterns observed in the *in vivo* cohorts. Pairwise comparisons between cohorts revealed that male and female MPMCs are different in their protein translation and peptide metabolism pathways while aging may be changing their metabolic processes. Future analysis will be conducted on the specific proteins using function blocking *in vitro*, *in vivo*, and *ex vivo* assays to evaluate metastatic potential. The results will be evaluated to generate **an integrated portrait of the contribution of gender and age to CRC interaction with MPMC in peritoneal metastasis**.

***Basic Science*** ***Graduate Student***

# POSTER #42

## AN INVESTIGATION INTO PEPTIDERGIC SENSORY NERVES AND INTERLEUKIN-1 IN PROSTATE CANCER

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### Background

The prostate cancer (PCa) tumor microenvironment (TME) contains a variety of neurotrophic growth factors and inflammatory cytokines, including nerve growth factor (NGF) and interleukin-1 (IL-1), that promote the growth of nerve fibers into the TME. The prostate is densely innervated by sympathetic and parasympathetic nerves that regulate smooth muscle tone and glandular secretion. In prostate cancer, sympathetic and parasympathetic nerves promote tumor growth and migration. Neuropeptides from peptidergic sensory nerves (PSN), such as tachykinins and calcitonin gene-related peptide (CGRP), are also present in the prostate, suggesting the presence of PSN. Moreover, PSNs are critically important for a myriad of physiological processes other than nociception, including immune regulation, vascular patterning, stem cell regulation, bone homeostasis, and tissue repair and recovery. However, no studies have selectively examined the functional role of sensory nerves in the prostate and in prostate cancer. Therefore, we hypothesize that IL-1 in the tumor microenvironment can alter the function of sensory neurons to subsequently alter prostatic function and/or prostate cancer progression.

### Methods

Primary cultures of rat dorsal root ganglion (DRG) neurons were treated for 24 or 72 hours with 0, 1, 10, or 30 ng/mL of IL-1α and the release and total content of CGRP were assessed. Wide-field immunofluorescence (IF) imaging was used to determine IL-1R1 expression on sensory nerves. To evaluate the microarchitecture of PSNs, prostates were harvested from transgenic mice expressing EGFP driven by the CGRP promoter (*Calca*-fEGFP) and fixed with 4% paraformaldehyde. Tissues were immunolabeled and processed through a modified ethyl cinnamate-based optical tissue clearing protocol and imaged by confocal microscopy.

### Results

In primary cultures, IL-1α treatment enhanced stimulated CGRP release in a time and concentration-dependent manner while the total CGRP content of CGRP remained unchanged. IF imaging revealed that IL-1R1 is expressed on neuronal soma and on axons as discrete puncta. In prostate tissue, continuous, tortuous $GFP^+/CGRP^+$ nerves fibers are seen in 50–100μm thick volumes. Punctate CGRP signals were dispersed along continuous $GFP^+$ fibers indicative of large, peptidergic dense core vesicles in PSNs.

### Conclusions

Our data show that IL-1R1 is expressed on sensory nerves and that IL-1α sensitizes PSNs, increasing neuropeptide release. While previously thought to be sparsely distributed, 3D imaging with cleared tissues has enabled us to illustrate the abundant and highly-organized microarchitecture of PSN-neuronal fibers interwoven around prostatic acini, proximal to epithelial glands suggesting that PSNs play a role in normal prostatic function.

Taken together, our data show that peptidergic sensory nerves are closely associated with prostatic acini and are sensitized by IL-1, supporting the notion that the inflammatory prostate TME can functionally alter the release of neuropeptides from PSN. Determining the implications this has on PCa proliferation and metastasis warrants further investigation.

***Basic Science*** ***Graduate Student***

# POSTER #43

## CONDITION-SPECIFIC GENE CO-EXPRESSION NETWORK ANALYSIS REVEALS COPY NUMBER VARIATIONS ASSOCIATED WITH KRAS MUTATION STATUS IN COLON CANCER

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Background: KRAS mutation happens in 30%-40% colorectal cancer, which is associated with more aggressive disease poor prognosis. To understand the impact of KRAS mutations in colorectal cancer, we carried out a condition-specific gene co-expression network analysis (GCNA). Our goal is to identify co-expressed gene modules that are specific to the colorectal cancer with KRAS mutations or without KRAS mutations. Our study on the condition-specific gene co-expression networks brings new biology understanding of the biological processes and structural variations associated with KRAS mutations.

Methods: Our analysis was conducted on the TCGA colorectal cancer (COAD) RNA-seq data set using the lmQCM algorithm. GCNA was separately applied to the KRAS mutant and non-mutant sample group. Gene modules with at least 20 genes were selected. The modules in one group containing less than 20% overlap with all modules in the other group is considered condition specific. Gene set enrichment analyses were carried out using TOPPGene against chromosome cytobands and canonical pathways.

Results: We have identified 25 of co-expressed gene modules for the KRAS mutant group and 30 for the non-mutant group, out of which 11 are specific to the KRAS mutant group and 14 are specific to the non-mutant group. Enrichment analysis on these condition-specific gene modules identified 9 (out of 11) are significantly enriched on different cytobands for the KRAS mutant group while all 14 are significantly enriched on different cytobands for the non-mutant group. Among the enriched cytobands, 5q12.3, 17p13.2, and 4p16.3 are unique for the KRAS mutant group and 20q13.33, 8q24.3, Xq21.2, Xq28, and 18q21.1 is unique for the non-mutant group. We selected gene from these cytobands and inspected their copy number readings between the two groups using cBioPortal. We observed consistently larger range of copy number variances in the group to which the original gene modules were specific.

Discussion and conclusion: Our observation suggested that there are different mechanisms related to genome stability with respect to KRAS mutation status. For the KRAS mutant group, the only uniquely enriched cytoband contains the important colon cancer gene APC while for the non-mutant group, the enriched cytobands cover important genes such as MYC (8q24) and TP53 (17p21). Interestingly chromosome X is highly enriched in the non-mutant group, suggesting the gender as a factor. Overall, our analyses have led to new hypotheses regarding the relationships between KRAS mutation status in colon cancer and the other genetic variations and biological processes.

***Basic Science*** ***High School Trainee***

# POSTER #44

## IL-1R1 DRIVES LEUKEMOGENESIS INDUCED BY TET2 LOSS

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Loss of the *ten-eleven translocation methylcytosine dioxygenase 2*(*Tet2*) gene, which is commonly mutated in hematological malignancies, dysregulates inflammatory pathways, including the interleukin-1 (IL-1) pathway. Roles for IL-1 signaling have been reported in terminally differentiated hematopoietic cells and in non-cell autonomous contexts. However, our group demonstrated that inhibition of inflammatory pathways can suppress clonal hematopoiesis, indicating potential direct roles for hematopoietic stem and progenitor cells (HSPCs) in inflammation. As *TET2* mutations are often present in HSPCs and provide these cells with a competitive advantage, dysregulation of the IL-1 pathway in HSPCs may contribute to leukemogenesis. Mutations in the *TET2* gene are detected in acute myeloid leukemia (AML). Similarly, *Tet2*$^{-/-}$mice and recipients of their bone marrow (BM) exhibit splenomegaly, monocytosis, extramedullary hematopoiesis, and expansion of Lin$^{-}$;Sca1$^{+}$;c-Kit$^{+}$ (LSK) cells. Based on these findings, we hypothesized that loss of the *Il-1r1* gene would rescue the hematological abnormalities associated with *Tet2* deficiency at the HSPC level. To investigate this possibility, we generated *Tet2*$^{-/-}$*;Il-1r1*$^{-/-}$ mice and analyzed mature and immature hematopoietic cells in these mice by flow cytometry. *Il-1r1* loss rescued the leukemic phenotypes associated with *Tet2* inactivation. Specifically, *Tet2*$^{-/-}$*;Il-1r1*$^{-/-}$ mice exhibited reduced levels of various myeloid cells, including neutrophils, monocytes, and eosinophils, and restored the levels of multiple suppressed lymphoid cell types. *Il-1r1*expression was strongly detected in HSPCs. Elevated levels of LSKs, long-term hematopoietic stem cells (LT-HSCs), short-term HSCs (ST-HSCs), multipotent progenitors 3/4 (MPP3/4), common myeloid progenitors, and Lin$^{-}$;Sca1$^{+}$ cells and suppressed levels of Lin$^{-}$;CD127$^{+}$ cells were corrected in *Tet2*$^{-/-}$*;Il-1r1*$^{-/-}$mice, compared to *Tet2*$^{-/-}$ mice. Competitive transplantation showed similar alleviations of the myeloid, lymphoid, and HSPC phenotypes, supporting a cell autonomous role for *Il-1r1* in HSPCs. Loss of *Il-1r1* in the BM also corrected the red cell width distribution (RDW-CV), a marker of inflammation, and elevated levels of several pro-inflammatory cytokines and chemokines, including tumor necrosis factor-alpha and interferon-gamma-inducible genes. Pediatric and adult AML patients with higher *IL-1R1* expression showed reduced survival, and worse survival outcomes were observed in specific subsets of adult AML patients. In summary, we have shown that loss of *Il-1r1* in *Tet2*$^{-/-}$HSPCs rescued several abnormalities associated with *Tet2* deficiency, including the elevation of LSK cells, the pro-inflammatory state, and the myeloid-lymphoid imbalance, and have demonstrated the potential clinical relevance of *IL-1R1* expression in AML. Collectively, these findings underscore a potential therapeutic role for IL-1 signaling in hematological malignancies at the stem-cell level.

***Basic Science*** ***MD/PhD or MSTP student***

# POSTER #45

## EVALUATING THE EFFECTS OF TARGETED DRUG THERAPIES FOR 8Q24.3 AMPLIFIED BREAST CANCER

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**Background/Objective:**

Cancer studies have helped us understand recurrent chromosome abnormalities leading to tumor progression. One such recurrent genomic aberration found in breast cancer is chromosome 8q24.3 (Chr. 8q24.3) amplification. We identified Tonsoku Like, DNA Repair Protein (TONSL) located within this amplicon as an immortalizing oncogene, with TONSL-overexpressing cells exhibiting distinctively upregulated homologous recombination (HR). Further experiments have shown that cancer cells with TONSL amplification are sensitive to the FACT inhibitor CBL0137, which is in early phase of clinical development. Based on known functions of TONSL in promoting dsDNA repair, we hypothesized that drug combinations targeting multiple pathways of DNA repair would synergize to kill chromosome 8q24.3 amplified breast cancer cell lines.

**Methods:**

Chr.8q24.3-amplified breast adenocarcinoma cell line TMD436 was utilized in this study. Cells were treated with various drugs targeting DNA repair pathways such as ATR inhibitor (VE-822), PARP inhibitor (Talazoparib), and PI3K inhibitor (BYL719). Cell proliferation rates were measured using bromodeoxyuridine incorporation ELISA.

**Results:**

Thus far, the use of $PI3K_i$ and $PARP_i$ combination has had an additive effect - the combined effect of the two drugs is equal to the sum of the effect of each agent given alone. The effect of $ATR_i$ and $PARP_i$ combination was antagonistic.

**Conclusion/Potential Impact:**

This study establishes the potential feasibility of using DNA repair signaling inhibitors in the treatment of TONSL-overexpressing breast cancer. Future studies extending the range of drug concentrations and newer combinations may ultimately lead to translation of these drugs to in vivo models and clinical trials. By

targeting multiple DNA repair pathways, similar approaches may sensitize patients to lower doses of chemotherapeutics, thus decreasing unwanted side effects. Moreover, negative data concerning the $ATR_i$/$PARP_i$ combination helps to further refine which drugs may be used to treat Chr. 8q24.3 amplified tumors and to understand signaling pathways active in TONSL-overexpressing breast cancer.

***Basic Science*** ***Medical Student***

# POSTER #46

## IMAGE-BASED MICROBIOME PROFILING DIFFERENTIATES GUT MICROBIAL METABOLIC STATES

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Bile acids are intestinal metabolites that are biotransformed into diverse secondary bile acids to aid with digestion and absorption. However, once modified by the gut microbiome, they can produce serious health implications including colorectal cancer risk. We hypothesized that this bile acid metabolism is reflected in bacterial cell morphologic changes. To test this hypothesis, we anaerobically cultured and generated light microscopy images of Clostridium scindens in media containing 100 μM cholic acid (a known substrate in the production of the carcinogenic bile acid deoxycholic acid), C. scindens in media containing NaCl (a positive control; 1% NaCl is shown to cause shrinkage through osmosis in bacterial cells), and C. scindens in media alone (negative control) (8 images per group; 500 bacterial cells per image). We developed an image-based model using MicrobeJ (an ImageJ plug-in developed for analysis of bacterial images) by using smoothed particle contours and a skeletonization algorithm adjusting area, length, width, and circularity parameters to accurately detect cells. We observed a significant difference in shape descriptor analysis between curvature of the end points and center of the medial axes, width of the medial axes, ratio between the major and minor axes of the cells, ratio between area and convex area, angularity, roundness ($4 \times \text{area} / \pi \times \text{major axis}2$), length of the medial axes, circularity ($4\pi \times \text{area} / \text{perimeter}2$), and perimeter of the outside boundary between Clostridium scindens with and without the presence of cholic acid ($p < 10\text{-}5$ for all comparisons; Welch's two-tailed t-test). Of note, this represented a larger difference than the delta between the two controls. Our data demonstrate that image-based analysis can enable detection of cellular morphologic differences of C. scindens based on metabolic profile with respect to bile acids. In principle, this approach could be expanded to other bile acids and/or beyond bile acid metabolism to identify bacterial metabolic behaviors of interest (e.g. assessing or predicting effects of clinically relevant compounds targeting the microbiome) or aid with statistical modeling screening for colorectal cancer.

***Basic Science*** ***Medical Student***

# POSTER #47

## A CANCER CELL'S TOOLBOX FOR CONQUERING OTHER ORGANS: DISCOVERING AND COMBATING THE SECRETOME OF A METASTASIS CAPABLE CANCER CELL

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**Background and Hypothesis:**

Previous studies have recognized that abnormal signaling by RAS oncogenes is predominantly observed in metastatic breast cance. A hypothesis was developed: the cancer cells with abnormal RAS genes release protein factors into the blood stream which can reorganize the signaling of non-breast tissue in a way that mimics breast tissue therefore making this organ prone to metastasis. These RAS-dependent factors can be targeted therapeutically to decrease metastasis.

**Experimental Design:**

Three cell lines were plated for the experiment: KTB-hTERT immortalized cell line as the control line, KTB-hTERT transformed derivatives TKTB RAS + SV40, which forms metastatic adenocarcinomas in NSG mice, and TKTB PIK3CA + SV40, which forms non-metastatic adenocarcinoma in NSG mice. Three Western Blots were conducted with protein readings for phospho-PAK4, PAK4, phospho-PIK3CD, and PIK3CD. These experiments were done to begin to test the hypothesis that phospho-proteome unique to RAS transformed cells regulate secretome with an effect on distant organs. These cell lines were examined for sensitivity to PIK3CD inhibitor Idelalisib and MEK1/MEK2 inhibitor Trametinib, which mediates signals downstream of RAS that regulate PIK3CD, using BrdU-incorporation ELISA proliferation assay.

**Results:**

Through the western blot analysis, it was consistently shown that there is a significant increase in the production of phospho-PIK3CD and PIK3CD in RAS over PIK3CA which shows that PIK3CD could be a protein that leads to metastasis of RAS transformed cells. Idelalisib did not display activity in any cell lines. Trametinib showed decreased growth of all cell lines and RAS-transformed cells were less sensitive to the drugs suggesting hyperactivation of this pathway in RAS-transformed cells.

**Conclusion and Impact:**

This study brings breast cancer research closer to pinpointing which proteins, in this case PIK3CD, can be targeted to decrease metastasis. The development of a drug that is specific to PIK3CD should be pursued to discover a treatment that decreases breast cancer metastasis.

***Basic Science*** ***Medical Student***

# POSTER #48

## SYNTHESIS AND BIOLOGICAL EVALUATION OF CARDIAC GLYCOSIDES FOR CANCER THERAPY BY TARGETING THE DNA DAMAGE RESPONSE

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Cardiac Glycosides (CGs) are bioactive compounds originally used to treat heart diseases, but recent studies have demonstrated their anti-cancer activity. We previously demonstrated that *Antiaris toxicaria* 2 (AT2) possesses anticancer activity in KRAS mutated lung cancers via impinging on the DNA damage response (DDR) pathway. Towards developing this class of molecules for cancer therapy, herein, we report a multi-step synthetic route utilizing k-strophanthidin as the initial building block for determination of structure-activity relationships (SARs). A systematic structural design approach was applied that included modifications of the sugar moiety, the glycoside linker, stereochemistry, and lactone ring substitutions to generate a library of *O*-glycosides and *MeON*-neoglycosides derivatives. These molecules were screened for their anti-cancer activities and their impact on DDR signaling in KRAS mutant lung cancer cells. These results demonstrate the ability to chemically synthesize CG derivatives and define the structure activity relationships to optimize AT2 as a cancer therapeutic

***Basic Science*** ***Post-Doctoral/Medical Fellow***

# POSTER #49

## INTEGRIN LINKED KINASE ACTIVATES BETA-CATENIN AND PROMOTES THE OVARIAN CANCER STEM CELL PHENOTYPE

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**Background:** Our research investigated the therapeutic potential of integrin-linked kinase (ILK) as a key target regulated by fibronectin (FN) deposition and integrins clustering in pre-clinical ovarian cancer (OC) models. Intraperitoneal (ip) metastases and chemoresistance represent major causes of poor clinical outcome in OC. Thus, identifying new therapeutic targets is crucial for developing treatments for chemoresistant OC patients and prolonging survival. One critical factor regulating the development of ip metastasis is the formation of spheroids that serve as the vehicle for tumor dissemination in the peritoneum, protecting cells from anoikis induced by stress in the extracellular compartment. Studies *in vitro* and *ex-vivo* models indicated that OC tumor cells increased extracellular matrix (ECM) proteins, which include FN, and cell adhesion molecules, such as integrins, that mediate the proliferative ability and viability of OC spheroids. Mechanistically, the interaction between FN and the integrins recruits the adaptor protein ILK that serves as docking site for downstream intracellular molecules, modulating several cancer cells associated processes, including survival, proliferation, and migration. However, limited number of studies are available on the functional role of ILK in the initiation, progression, and chemoresistance of OC. We hypothesized that development of ILK blocking strategies will target the chemoresistant cancer stem cells (CSCs) and their proliferation as spheroids.
**Methods:** We compared ILK, FN, and integrin beta1 expression in primary OC cells grown as spheroids and monolayers and in chemoresistant versus sensitive OC cell lines by q-PCR and western blot (WB). RNA interference or pharmacological inhibition of ILK through compound 22 (cpd-22) was evaluated on spheroid assay and colony formation. Combinatorial carboplatin and cpd-22 treatment was assessed on spheroid assays and by WB of pro-apoptotic proteins.
**Results:** Our results demonstrated increased FN, integrin beta1, and active-p-ILK at Ser246 in spheroids and chemoresistant OC cells. Mechanistically, the integrin beta1-ILK transduction signal inactivated GSK-3alpha/beta by phosphorylation at Ser21/9 and amplified Wnt-3A signals with increased beta-catenin-TCF/LEF1 transcriptional activity. In addition, ILK enhanced the resistance to apoptosis induced by chemotherapy by direct activation of p-Akt at Ser473. ILK blockade by either RNAi or cpd-22 treatment in combination with carboplatin led to a significant decrease in the expression levels of stemness-related genes, spheroids, and colony formation. Mechanistically, the combination of carboplatin and ILK blockade decreased the oncogenic beta-catenin pathway and inhibited p-Akt at Ser473 with consequent increased levels of cleaved-caspase-3/7 compared to single agent alone, indicating sustained apoptotic damage.
**Conclusions:** ILK is strongly linked to chemoresistance and is regulated by the oncogenic beta-catenin pathway. Our data suggest that ILK inhibition along with carboplatin treatment could be a potential combination therapy in OC.

***Basic Science*** ***Post-Doctoral/Medical Fellow***

# POSTER #50

## CANCER DERIVED EXOSOMES AS POTENT DIAGNOSTIC AND THERAPEUTIC BIOMARKERS FOR SOLID TUMORS

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Exosomes characterize a subtype of extracellular vesicle (EV) that is released through the fusion of multivesicular bodies with the plasma membrane. Previous research has shown that exosomes derived from cancer cells can act as significant biomarkers for the diagnosis and prognosis of cancer. However, the link between the protein and lipid makeup of EVs and their source cells and function is not well understood. Therefore, the main objective of this study was to reveal the proteomic and lipidomic landscape of exosomes derived from cancer and the normal cell line to identify potential therapeutic targets. Here, we conducted comprehensive proteomic and lipidomic analyses of exosomes from breast cancer (MDAMB231, MCF7), lung cancer (A549, NCI-H460), and prostate cancer (PC3, C4/2B) cell lines as well as their related healthy primary cell lines. Exosomes were isolated by the size exclusion chromatography method from the cell line supernatant. The isolated exosomes were characterized by nanoparticle tracking analysis and transmission electron microscopy. Further, we performed proteomics and lipidomic analyzes to identify specific biomarkers. We identified the expression levels of exosomal proteins by liquid chromatography-tandem mass spectrometry (LC-MS/MS) based on label-free quantification and lipidomic targets were identified by quadrupole time-of-flight LC/MS. Multiple bioinformatics packages were used to analyze the proteomic and lipidomic data to identify significant proteins and lipid profiles from different solid tumors. We also identified important mutated proteins from our data to predict liquid biopsy-based neoantigens. Isolated exosomes were in the size range of 60-80 nm with a concentration of $10^{11}$ particles/mL. A total of 3194 proteins and 4000 lipid molecules were identified from 9 cell line-derived exosomes. Several proteins and lipid molecules significantly vary in cancer-derived exosomes compared to those from a normal cell line. For instance, we observed that the CDKs (Cyclin-dependent kinases), MCM (Minichromosome maintenance), EMT (Epithelial-mesenchymal transition) and HSP (*Heat shock proteins*) proteins family were, in general, more abundant in exosomes from solid tumor cell lines than from normal cell line; LPC (Lysophosphatidylcholine) and lipoprotein lipase (LPL), Sphingomyelin (SM) and Phosphatidylcholines (PC) were significantly higher in cancer-derived exosomes than normal ($p < 0.05$). Most importantly, we found significantly mutated proteins such as mucin 1 (MUC1), isocitrate dehydrogenase 1 (IDH1), apolipoprotein B (APOB), and FAT atypical cadherin 4 (FAT4), which provide a proof of concept for liquid biopsy-based neoantigen. Our results disclose significant proteins and pathways involved in intercellular communication and an extraordinary arrangement of lipids into exosomes which may depend on their origin. Based on our knowledge this is the first comparative study of the proteomes and lipidomic profile of the solid tumor and normal cell line-derived exosomes. The comprehensive molecular analyses will contribute to our understanding of exosome biogenesis, and the results may have potential implications for biomarker discovery for solid tumors.

***Basic Science*** ***Post-Doctoral/Medical Fellow***

# POSTER #51

## TUMOR MICROENVIRONMENT MEDIATED CHROMATIN CHANGES AND C-JUN INDUCTION REGULATES OVARIAN CANCER METASTASIS

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Ovarian cancer (OC) is the primary cause of death from gynecologic malignancies and high-grade serous OC (HGSOC) is its most prevalent and lethal subtype. Most ovarian cancer patients are diagnosed at a late stage with extensive metastasis, which contributes to the high mortality rate. Ovarian cancer predominantly undergoes transcoelomic metastasis and the omentum – a large fat pad in the peritoneal cavity – is the most common site of metastasis. However, the regulation of OC metastasis, especially metastatic colonization, which is the rate-limiting step, is still poorly understood. During metastatic colonization, cancer cells must first successfully adapt to the new microenvironment before they can eventually develop into the metastatic tumor. This requires productive cross-talk between the OC cells and the microenvironment of the metastatic site, resulting in adaptive changes in gene regulation. Transcription factors (TFs) and epigenetic changes induced by the metastatic microenvironment would be expected to play a key role. Using an organotypic 3D culture model mimicking metastatic colonization of the omentum, combined with the endpoint analysis of matched primary tumors and metastases from HGSOC patients, we identified the c-Jun as a key transcription factor increased in metastasizing OC cells when they interacted with the tumor microenvironment. We also observed that the metastatic microenvironment induces changes in chromatin in the cancer cells when we performed ATAC-seq in HGSOC cells seeded on the 3D omentum culture model. The top TFs predicted to bind to the newly open chromatin regions were Fos and c-Jun, further confirming the important role of c-Jun during metastasis. Fos and Jun combine to form AP-1 to regulate transcription. Therefore, we have performed a c-Jun CUT&RUN in HGSOC cells seeded on the 3D culture model and overlapped the results with the ATAC-seq data, to identify the direct targets of c-Jun that are induced by microenvironmental signals. HGSOC cells co-culture with microenvironment cells and conditioned medium (CM) experiments revealed that paracrine signals from mesothelial cells and cancer-associated fibroblasts (CAFs) regulated c-Jun. Mass spectrometric analysis of the secretome of omental mesothelial cells (HPMC), CAFs, and normal omental fibroblasts (NOF), was used to identify the key paracrine factors involved. Treatment with DNMT, EZH2, or HDAC inhibitors increased expression of c-Jun and its targets, confirming the role of chromatin remodeling in the regulation of their expression by microenvironmental signals. Functional studies revealed that c-Jun regulated migration, invasion through the outer layers of the omentum, and colony formation on the omentum. Moreover, knocking out c-Jun significantly decreased metastasis in a mouse HGSOC xenograft model. Taken together, our studies reveal the novel phenomenon of microenvironment-induced upregulation of c-Jun, combined with the microenvironment-induced opening of the chromatin of certain c-Jun binding sites, which together regulate HGSOC metastatic colonization through specific transcriptional targets. Since microenvironment-induced upregulation of c-Jun and chromatin changes enabling transcription of its targets is essential for OC metastasis, targeting it or the cross-talk with the microenvironment may be a novel strategy against this deadly cancer.

***Basic Science*** ***Post-Doctoral/Medical Fellow***

# POSTER #52

## SREBP MEDIATED LIPID BIOSYNTHESIS IS A POTENTIAL TARGET FOR TREATING GC DERIVED B CELL LYMPHOMA

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Diffuse large B cell lymphoma (DLBCL), derived from aberrant germinal center (GC) reactions, is a genetically heterogeneous and highly aggressive malignancy. Although timely treatments with combined therapies have improved the survival, relapse occurs in 30-40% of patients and can be fatal. Here, we provide a new strategy for treating DLBCL by targeting lipid biosynthesis. Sterol regulatory element binding proteins (SREBPs) are transcription factors playing a central role in coordinating de novo cellular lipogenesis. We discovered that SREBP2 protein expression is elevated in a subset of normal human GC B cells and is highly expressed in both ABC type and GCB type human lymphoma samples. Inhibiting SREBP activation or downstream signaling by specific inhibitors (Fatostatin and Simvastatin) significantly inhibited the proliferation of mouse and human DLBCL cells lines in vitro. Fatostatin treatment also inhibited tumor growth of Karpass422 cells in an animal model. These data suggested a therapeutic potential of targeting lipid biosynthesis for treating DLBCL.

***Basic Science*** ***Post-Doctoral/Medical Fellow***

# POSTER #53

## KU-DNA BINDING INHIBITORS MODULATE THE DNA DAMAGE RESPONSE IN RESPONSE TO DNA DOUBLE-STRAND BREAKS

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The DNA-dependent protein kinase (DNA-PK) plays a critical role in the DNA damage response (DDR) and non-homologous end joining (NHEJ) double-strand break (DSB) repair pathways. Consequently, DNA-PK is a validated therapeutic target for cancer treatment in certain DNA repair-deficient cancers and in combination with ionizing radiation (IR).

We have previously reported the discovery and development of a novel class of DNA-PK inhibitors with a unique mechanism of action, blocking the Ku 70/80 heterodimer interaction with DNA. These Ku-DNA binding inhibitors (Ku-DBis) display nanomolar activity *in vitro*, inhibit cellular DNA-PK, NHEJ-catalyzed DSB repair and sensitize non-small cell lung cancer (NSCLC) cells to DSB-inducing agents. In this study, we demonstrate that chemical inhibition of the Ku-DNA interaction potentiates the cellular effects of bleomycin and IR via p53 phosphorylation through the activation of the ATM pathway.

This response is concomitant with a reduction of DNA-PK catalytic subunit (DNA-PKcs) autophosphorylation at S2056 and a time-dependent increase in H2AX phosphorylation at S139.

These results are consistent with Ku-DBis abrogating DNA-PKcs autophosphorylation to impact DSB repair and DDR signaling through a novel mechanism of action, and thus represent a promising anticancer therapeutic strategy in combination with DNA DSB-inducing agents.

***Basic Science*** ***Post-Doctoral/Medical Fellow***

# POSTER #54

## MECHANISMS OF MUTANT P53 TARGETING TO THE GENOME

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Mutations in genes often result in tumor formation, especially when these mutations occur in tumor suppressors. TP53 is a tumor suppressor that is frequently mutated in cancer, with mutations in this gene occurring in nearly 50% of all cancer cases. Once mutated, p53 loses its tumor suppressive function while simultaneously gaining oncogenic function. One of the functions that mutant p53 loses is the ability to directly bind to chromatin, however, it has been reported that mutant p53 can still affect the transcriptome of cancer cells via interactions with other transcription factors. One of these interacting partners is ETS2. ETS2 belongs to the ETS transcription factor family, which has 28 family members. This family is characterized by their affinity for an ETS binding site (EBS). EBS's are present in 50% of all mutant p53 occupied promoters. Other ETS family members have also been linked to mutant p53 but these interactions have either been deemed as weak (ETS1) or have yet to be identified as direct (ERG).

To determine which ETS proteins interact with mutant p53, I conducted affinity pull-down assays using purified ETS proteins and purified mutants of p53. My data shows that several ETS proteins interact with mutant p53 better than ETS2. I then sought to determine which residues are important for this interaction through truncation studies in which I used purified truncations of ETS proteins and purified mutants of p53. I found that ERG, one protein that strongly interacted with mutant p53, had two interaction interfaces. This may explain why the interaction is strong.

My next step was to determine which ETS proteins are responsible for the targeting of mutant p53 to the genome. To address the requirement for ETS to recruit mutant p53 to chromatin I performed chromatin immunoprecipitation sequencing (ChIP-Seq) studies in the presence or absence of different ETS factors to determine differences in mutant p53 binding. For these studies I knocked down ETS2 or ERG prior to performing p53 ChIP-Seq to determine differences in p53 binding to chromatin under these conditions. My analyses of these data indicate that each of the conditions resulted in different p53 binding patterns in the ChIP-Seq and that there is a requirement for ETS in mutant p53 binding.

My studies have demonstrated that ETS proteins interact with mutant p53 and that this interaction seems to be required for mutant p53 binding to the genome. My future work will test phenotypes related to ETS/mutant p53 interactions. Ultimately, if ETS/mutant p53 interactions are deemed important for oncogenic function, these will be attractive targets for future drug development.

***Basic Science*** ***Post-Doctoral/Medical Fellow***

# POSTER #55

## ACTIVATION OF THE INTEGRATED STRESS RESPONSE (ISR) PATHWAYS IN RESPONSE TO REF-1 INHIBITION IN HUMAN PANCREATIC CANCER AND ITS TUMOR MICROENVIRONMENT

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by a profound inflammatory tumor microenvironment (TME) with high heterogeneity and metastatic propensity, metabolic reprogramming, and extreme hypoxia. The integrated stress response (ISR) pathway features a family of protein kinases that phosphorylate eukaryotic initiation factor 2 (eIF2) and regulate translation in response to diverse stress conditions, including hypoxia and nutrient deprivation. Phosphorylation of eIF2 reduces global translation, but paradoxically increases translation of select mRNA transcripts, such as the transcription factor ATF4 which regulates gene expression to promote either stress adaptation and cell survival or apoptosis. Apurinic/Apyrimidinic endonuclease/reduction-oxidation factor 1 (APE1/Ref-1 or Ref-1) is a dual function enzyme for DNA repair and redox signaling. The redox function of Ref-1 directly regulates multiple transcription factors including HIF-1α, STAT3, and NF-κB, which are highly active in the PDAC TME. Our previous work demonstrated that eIF2 signaling pathways were profoundly affected in response to Ref-1 knockdown in human PDAC cells. However, the mechanistic details of the crosstalk between Ref-1 redox signaling and regulation of ISR pathways are unclear.

We evaluated the effects of loss of Ref-1 on ISR signaling in human PDAC cells and cancer-associated fibroblasts (CAFs). Following Ref-1 knockdown, induction of the ISR response was observed under normoxic conditons, while hypoxic conditions were sufficient to activate ISR irrespective of Ref-1 levels. Inhibition of Ref-1 redox activity induced ATF4 transcritptional activity, activated the ISR kinase PERK, and increased expression of p-eIF2 and ATF4 in a concentration-dependent manner in multiple human PDAC cell lines.

Using the PERK inhibitors HC-5404 and GSK2656157, in monolayer and 3D co-culture systems, we demonstrated that CAF19 cells are more sensitive to PERK inhibition in 3D culture compared to tumor cells. Interestingly, treatment with HC-5404 at high concentrations resulted in activation of the alternative ISR kinase GCN2 and induced levels of p-eIF2 and ATF4 in both tumor cells and CAFs. Combination treatment with inhibitors of Ref-1 and PERK enhanced cell killing effects in both human PDAC tumor lines and CAF19 line in 3D culture. However, the effects were greater on CAF lines as compared to tumor lines and were only observed in the 3D co-cultures. Altogether, we demonstrate that the ISR is active in multiple PDAC lines following targeting of Ref-1 redox signaling. Our combination studies demonstrate greater effects when the ISR is activated but only in physiologically relevant 3D co-culture models, suggesting that Ref-1 redox signaling may play a critical role in pro-survival and that inhibition of Ref-1 signaling induces cell death through ISR signaling pathways. This work suggests that the combination of Ref-1 redox signaling blockade with ISR activation could be a novel therapeutic strategy for PDAC treatment, and that the model system utilized can greatly affect the outcome of these targeted agents.

***Basic Science*** ***Post-Doctoral/Medical Fellow***

# POSTER #56

## INSIGHTS INTO HIGHLY FUNCTIONAL HUMAN HEMATOPOIETIC STEM CELLS FROM 27-YEAR OLD CRYOPRESERVED UMBILICAL CORD BLOOD

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Umbilical cord blood (CB) is a promising source of hematopoietic stem cells (HSCs) and progenitor cells (HPCs) for hematopoietic cell transplantation, a life-saving treatment for malignant and non-malignant hematologic disorders. CB is cryopreserved following collection, allowing for the amassment of nearly 5 million CB units in private and public banks. It is critical to understand how long CB can be stored with efficient recovery of engraftable HSCs. It is also important to explore what, if any, specific molecular profiles can predict which units will efficiently engraft regardless of other variables, such as age of the unit. To study effects of long-term cryopreservation on HSC function, we obtained 3 CB units that had been cryopreserved for >27 years. The CB units were thawed and total nucleated cells were recovered with 76-87% viability. Immunophenotyping assays showed that cryopreserved CB units had equivalent numbers of CD34+ cells, a commonly used parameter to predict utility in a clinical setting. Within the CD34+ population, cryopreserved CB when compared to fresh CB had on average equal or greater numbers of stringently immunophenotypically defined HSCs. Colony forming unit (CFU) assays demonstrated that cryopreserved CB units exhibited lower functional HPC numbers, yielding 27% the number of colonies compared to fresh CB, consistently with previous studies. In *ex vivo* analysis of HSC/HPC proliferative capacity, cryopreserved CB units showed similar capacity for CD34+ cell expansion in growth stimulating media compared to fresh CB. For *in vivo* functional HSC analysis, limiting dilution transplantation studies of CD34+ cells to sublethally irradiated immune-deficient mice revealed that 27-year old CB units contain HSCs with low to robust engraftment capacities. Two cryopreserved CB units had high repopulating frequency, a gold standard measure of HSC function, while the third had low repopulating frequency. Secondary transplants revealed secondary repopulation capacity, a measure of HSC self-renewal. Differences in repopulating capacity of the 27-year-old CB were not well predicted by any measured clinical parameter, thus we transcriptomically profiled the HSCs to gain insight into differences in functional competency between different CB units. RNA-seq revealed that molecular programs associated with self-renewal, oxidative stress responses, and quiescence and genes such as *TUSC3*, *TNF*, *MSX1*, *MYC,* and CXCL8 are enriched in high engrafting but not low engrafting CB HSCs. These data suggest functional HSCs with short- and long-term engrafting and sustained self-renewal capacity can be cryopreserved for at least 27 years. Thus, age itself should not be an exclusionary criteria for the selection of viable CB units for transplantation. However, criteria must be established that accurately reflects the transplantability of different CB units. We have shown that transcriptomic profiling can identify potential markers of functional engraftability for HSCs and provide insight into molecular programs that may be important for HSC function.

***Basic Science*** ***Post-Doctoral/Medical Fellow***

# POSTER #57

## CREATION AND CHARACTERIZATION OF NOVEL ADENOMYOSIS CELL LINES

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Endometriosis is a benign gynecologic disease characterized by the growth of endometrial-like tissue outside the uterus. A distinct subset of endometriosis, called adenomyosis, occurs when endometrial-like tissue is found within the uterine myometrium. Both traditional pelvic endometriosis and adenomyosis are risk factors for ovarian cancer, with persons with adenomyosis having a two-fold increased incidence of endometrioid ovarian cancer. Unfortunately, only one commercially available endometriotic epithelial-like cell line (12Z) and no adenomyosis cell lines exist. Cell line diversity is crucial for flexibility and optimization of research experiments. With IRB approval, the IUSCCC Biospecimens Collection and Banking Core ($BC^2$) procured endometrial tissue from a hysterectomy subject. Final surgical pathology revealed focal adenomyosis of the uterus. Sample processing included enzymatic digestion of the endometrial tissue, isolation of epithelial and stromal cells via the selective adhesion method, and fluorescence-activated cell sorting using epithelial cellular adhesion molecule (EpCAM) surface marker. Initial assessments of the primary EpCAM+ cell culture showed a slightly heterogeneous population exhibiting epithelial morphology with relatively no stromal cells. The primary cell culture displayed a rapid growth rate and wasimmortalized with human telomerase reverse transcriptase gene (hTERT) via retroviral transduction of a pLXSN-hTERT vector (kindly provided by Dr. Nakshatri, Indiana University). The designated tEEC21 cell line was 16-marker short tandem repeat (STR) profiled by IDEXX BioAnalytics andconfirmed to be genetically different from known cell lines. Immunofluorescence microscopy revealed that tEEC21 was positive for the epithelial markers, cytokeratin-7 and N-cadherin, and negative for the stromal marker, CD10. Current tEEC21 cell line characterization studies include monitoring two- and three-dimensional cell growth and morphology, proliferation and doubling time, karyotype, and response to steroid hormones. The establishment and delineation of this new cell line will serve as a research resource for adenomyosis and adenomyosis-associated gynecologic cancers.

***Basic Science*** ***Research Technician***

# POSTER #58

## INCREASED PIEZO1 EXPRESSION IS ASSOCIATED WITH WORSE CLINICAL OUTCOMES IN HORMONE RECEPTOR NEGATIVE BREAST CANCER PATIENTS

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**Background:** Piezo1 plays a crucial role in the human body as a mechanosensory ion channel, which notably transports $Ca^{2+}$ to the intracellular space. It has been demonstrated that Piezo1 is important in tissue development and regulating many essential physiological processes. However, its importance and role in malignancies, and specifically breast cancer, is not well understood. Past studies have suggested that Piezo1 plays a role in invasion and cancer progression; high levels of Piezo1 have been correlated with increased migration in breast cancer cells, chemo-resistance and invasion in gastric cancer cells, and increased invasion of osteosarcoma cells. In addition, high PIEZO1 expression levels were correlated with a worse prognosis in glioma patients. On the other hand, studies in lung cancer have attributed high Piezo1 levels to better patient outcomes. The impact of Piezo1 in breast cancer is not well characterized, therefore, the goal of this study was to determine the clinical relevance of Piezo1 in breast cancer.

**Methods:** Analysis of breast cancer data from The Cancer Genome Atlas (TCGA) provisional dataset was conducted to investigate PIEZO1 expression levels and correlation to survival, followed by confirmation in an independent dataset, GSE3494. We also performed supplementary gene set enrichment analysis (GSEA) through the Broad Institute's molecular signatures database (MSigDB), as well as analysis of immune cell levels in primary tumors from TCGA through a CIBERSORT algorithm.

**Results:** Our results demonstrate that PIEZO1 expression levels are higher in hormone receptor negative (HR-) cohorts than hormone receptor positive (HR+) cohorts. High PIEZO1 expression is correlated with a significant decrease in survival in HR- cohorts. GSEA shows that various signaling pathways associated with more invasive phenotypes and resistance to treatments, including epithelial-mesenchymal transition (EMT) and hypoxia, are enriched in high PIEZO1 HR- tumors. Our results also suggest that PIEZO1 expression levels inversely correlate with the levels of T cell infiltration into HR- breast cancer tumors, as there is a decrease in CD8+ and CD4+ T cells in samples from patients with high PIEZO1 expression based on mRNA analysis.

**Conclusion:** Our results show that PIEZO1 expression inversely correlates with survival in HR- breast cancer patients. The data suggests that this may be due to enrichment of EMT and hypoxia or the decrease of T cell infiltration. Further studies are needed to determine the mechanistic role of Piezo1 and mechano-signaling in breast cancer and the tumor microenvironment.

***Basic Science*** ***Research Technician***

# POSTER #59

## UTILIZING PP2A-EGFR CROSSTALK IN THERAPEUTIC STRATEGIES IN PDAC

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Pancreatic Ductal Adenocarcinoma (PDAC) ranks 4$^{th}$ in leading causes of cancer deaths and has one of the lowest five-year survival rates. In PDAC tumors, epidermal growth factor receptor (EGFR) signaling is aberrantly active and helps to aid in mutant KRAS$^{G12D}$ signaling, a mutation found in 95% of PDAC cases. Because KRAS$^{G12D}$ drug targets have yet to be developed, there is a need for new therapeutic strategies. Erlotinib, an EGFR inhibitor, is one of four FDA-approved targeted therapeutics for PDAC, but has provided minimal benefits alone in the clinical setting. Therefore, combination with other agents may be more effective, which has been validated in other cancer types.

PP2A is a major serine-threonine phosphatase that regulates KRAS and EGFR signaling by dephosphorylating downstream effectors. Small Molecule Activators of PP2A (SMAPs) are a promising cancer therapy to shut down KRAS- and EGFR- driven oncogenic pathways. A study in EGFR mutant lung cancer combined the SMAP DT-061 with EGFR inhibitors and found increased apoptosis in combination and decreased downstream oncogenic signaling.

Given the role of EGFR signaling in PDAC and the potential for PP2A activators, we sought to determine the relationship between these major signaling nodes in PDAC. By utilizing genetic and therapeutic methods, we have found a novel role for PP2A in EGFR regulation where PP2A activation elicits a feedback loop for EGFR activation. To understand this relationship, we have measured efficacy of PP2A activation combined with EGFR inhibition, demonstrating synergy in decreased cell viability and downstream EGFR signaling. Our findings suggest that PP2A crosstalk with EGFR is a critical signaling node in PDAC which can be therapeutically leveraged.

***Basic Science*** ***Undergraduate Student***

# POSTER #60

## INVESTIGATING THE RELATIONSHIP BETWEEN TGLI1-EXPRESSING GLIOBLASTOMA STEM CELLS AND ACTIVATED ASTROCYTES

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Glioblastoma (GBM) is a high-grade cancer of the brain which has significant clinical relevance due to the low prognosis and poor patient outcomes upon diagnosis. Cancerous tumors, such as GBM, typically arise from a multitude of biological modifications that alter the phenotype of normal cells. This research project investigates one such modification in a transcription factor called truncated glioma-associated oncogene homolog (tGLI1), an alternatively spliced isoform of GLI1. Unlike GLI1, tGLI1 expression is cancer-specific, and tGLI1 expression has been associated with tumor migration, invasion, and angiogenesis to a greater degree than GLI1 expression. High expression of tGLI1 compared to GLI1 has also been shown to promote a stem-cell-like phenotype in both GBM and breast cancer.

In the GBM tumor microenvironment, glial cells such as astrocytes have been shown to facilitate tumor progression. Upon an insult to the brain, such as cancer, astrocytes undergo changes in structure and function that result in an activated phenotype. Recently, published data showed tGLI1-expressing breast cancer stem-like cells activate astrocytes both *in vitro* and *in vivo*, which is believed to be the underlying mechanism of breast cancer brain metastasis. Preliminary data from our lab has further investigated the relationship between tGLI1 and activated astrocytes. Bioinformatic data from 165 GBM patients was analyzed using gene set enrichment analysis to show that two different activated astrocyte signatures are significantly enriched in patients with high tGLI1 activity. Furthermore, immunohistochemistry analysis of GBM patient samples showed a significant positive correlation between astrocyte activation and tGLI1 expression, but not GLI1 expression. Despite these findings, the underlying relationship between tGli1-positive glioma stem cells and astrocyte activation remains unclear and warrants further investigation.

***Basic Science*** ***Undergraduate Student***

# POSTER #61

## A META-ANALYSIS OF THE RELATIONSHIP BETWEEN ADVANCE CARE PLANNING AND THE CARE RECEIVED AT THE END-OF-LIFE AMONG PATIENTS WITH CANCER

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**Context:** Recent discourse on advance care planning (ACP) has underscored persistent ambiguity about its impact on end-of-life outcomes. The evidence base for ACP mostly relies on individual studies and narrative reviews. No meta-analysis exists to clarify these associations across a body of literature and in subsets of populations, such as patients with cancer.

**Objective:** To estimate the overall association between ACP and various forms of aggressive and comfort-focused end-of-life care outcomes among patients with cancer and to test moderators of these associations.

**Methods:** A systematic review and meta-analysis was conducted using PRISMA guidelines. Six databases were systematically searched for studies published since 1990 that examined the association between ACP and at least one end-of-life care outcome among samples of adult patients with cancer. For the meta-analysis, the effect size was the odds ratio (OR). Overall effects were computed using and inverse variance weighting and tests of moderation computed using weighted ANOVA-like models.

**Results:** Twenty-one articles met criteria, representing 33,541 participants and 68 effect sizes (54 aggressive, 14 comfort-focused end-of-life care outcomes). The association between ACP and aggressive end-of-life care outcomes was mixed. ACP was associated with significant reductions (by 29-52%) in the odds of chemotherapy, ICU admissions, hospital admissions, hospice use less than 7 days, hospital death, and composite measures of aggressive outcomes, whereas the odds of cardiopulmonary resuscitation, mechanical ventilation, and emergency department admissions were not significantly impacted. ACP type was a significant moderator of the impacts on hospital admissions outcomes, where studies that focused on the communication components of ACP had greater reductions in the odds of hospital admissions compared to other forms of ACP—60% versus 33% reduction. The association between ACP and comfort-focused end-of-life care outcomes was also mixed. ACP was associated with an increased odds of do not resuscitate orders by 1.52 times, whereas hospice use was not significantly impacted. Study design was a significant moderator of

the impacts on the hospice use outcomes, where studies with observational designs had significantly increased odds of hospice use, whereas those with experimental designs had non-significant impacts.

**Conclusion:** This meta-analysis demonstrates mixed evidence of the impacts of ACP on end-of-life care outcomes. However, the significant reduction in the odds of receiving chemotherapy, being admitted to the hospital or ICU, dying in the hospital, or using hospice for less than seven days combined with the increased odds of do not resuscitate orders provides evidence that ACP continues to hold value for patients with cancer at the end-of-life. This suggests that recent claims that ACP does not work are premature. Further efforts to clarify the unique impact of ACP on a broader set of outcomes beyond healthcare utilization and in other disease populations will continue to advance the field.

***Behavioral*** ***Faculty***

# POSTER #62

## IMPACT OF RELATIONSHIP CONCORDANCE ON BREAST CANCER SURVIVORS' PHYSICAL AND PSYCHOSOCIAL HEALTH

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### Background

Cancer survivors with strong relationship satisfaction and social support groups have been associated with better physical and psychosocial health, including depression, physical functioning, fatigue, and general quality of life (QOL). Given this, we examined the impact of relationship satisfaction and dyad relationship concordance between breast cancer survivors (BCS) and their partners with age-matched controls and their partners on different physical and psychosocial health metrics.

### Methods

Dyads of younger breast cancer survivors (<45 years old) and age-matched controls, and their partners were recruited as part of a larger, cross-sectional QOL study. Relationship concordance was measured by the ENRICH marital satisfaction score, with each dyad's score equaling the absolute value of the difference in satisfaction between survivor/control and their partner (lower score = greater concordance). Dependent variables for survivors/controls were partner social constraint, physical function, depression, fatigue, attention function (performing daily tasks), and sleep disturbance. Relationship satisfaction and relationship concordance were used as the primary independent variable for each of the six multiple linear regression models, while controlling for dyad category (younger vs. control), race, education, income, and age. Additionally, t-tests were used to compare relationship satisfaction, relationship concordance, and the six outcome variables between younger BCS and controls.

### Results

The sample consisted of 387 dyads (220 younger BCS and 167 controls). The mean relationship satisfaction across the sample was 52.4 (range: 8.5-88) and relationship satisfaction concordance ranged from 0-53.4, with mean of 10.3. The younger BCS had similar relationship satisfaction ($p=.498$) but significantly worse relationship concordance (11.12) than controls (9.08, $p=.050$). Within the multiple regression models, lower satisfaction was was significantly associated with increased partner social constraint ($p<.001$), worsening physical function ($p=.020$), increased depression ($p<.001$), increased fatigue ($p<.001$), decreased attention function ($p<.001$), and greater sleep disturbance ($p=.025$); while poor relationship concordance was significantly associated with partner social constraint ($p=.029$), increased depression ($p=.038$), and increased fatigue ($p=.006$).

### Conclusions

While we did not observe a difference in individual BCS and controls' ratings of their relationship satisfaction, we did see a significant difference in the relationship concordance, when factoring in partners' ratings. Looking at the impact on physical and psychosocial health, we see a significant association with relationship satisfaction and concordance with a majority of the outcomes. Although, we cannot determine a cause-effect relationship between relationship satisfaction and concordance and the physical and mental health metrics examined, this work does show the critical dynamic between BCS survivors and their partners. Given this evidence, it is vital to further examine this relationship, and for researchers to include partners when developing interventions targeted towards BCS.

# POSTER #63

## MUSCULAR ENDURANCE TESTING MAY NOT RELATE TO SELF-REPORTED FATIGUE IN AYA CANCER SURVIVORS

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BACKGROUND: Cancer related fatigue (CRF), or its treatment can lead to physical inactivity, reduced quality of life (QoL), and reduced functional capacity. Adolescent and young adult (AYA) cancer survivors are currently understudied and have increased cardiovascular and metabolic disease risk as they age. understanding how to reduce (CRF) and increase physical activity (PA) is paramount in this population. Understanding how PA can mitigate CRF effects may lead to a better QoL for AYA cancer survivors.

PURPOSE: To examine the relationship between self-reported CRF and muscular endurance/strength in AYA survivors.

METHODS: 29 AYA cancer survivors aged 15-39 (mean=27; M=7, F=22) were randomized into either an exercise (EX) or an attention control (AC) health coaching arm. Participants were assessed at baseline, 12-weeks, and 24 weeks. Measures included the Functional Assessment of Chronic Illness Therapy - Fatigue (FACIT-F), a 40-item questionnaire to assess CRF therapy, and a 60-second chair sit-to-stand test that evaluated muscular endurance and strength.

RESULTS: EX participants sit-to-stand scores increased by 3.23 repetitions on average, and FACIT-F scores improved by 1.3 points on average, however, the improvements were not statistically significant. AC participants' sit-to-stand scores and Facit-F scores worsened at the 12-week assessment (-1.25 repetitions and an increase in fatigue score of 1.89 points on average). These changes were also not statistically significant. There was generally no correlation observed between FACIT-F scores and the sit-to-stand scores when statistical analyses were performed. The correlation values for EX and AC groups respectively were $r=.213$, $r^2=.045$; $r=.165$, $r^2=.027$; and $r=.27$, $r^2=.073$.

CONCLUSION: These data suggest that the severity of CRF-related therapy may not correlate with muscular endurance tests such as the sit-to-stand test. Further research should explore higher-intensity exercise or longer interventions to that may reveal significant clinical outcomes in therapy-related fatigue and functional capacity in AYA cancer survivors.

***Behavioral*** ***Graduate Student***

# POSTER #64

## AN EVOLUTIONARY CONCEPT ANALYSIS OF SPIRITUALITY IN ADOLESCENTS AND YOUNG ADULTS WITH CANCER

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**An Evolutionary Concept Analysis of Spirituality in Adolescents and Young Adults with Cancer**

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**Purpose and Background/Significance:**Over the past 20 years, there has been an increased focus on the importance of spirituality to human health, including adolescent health outcomes. Yet, there is an absence of evidence-based programs to address the spiritual needs of patients and families – particularly in adolescent/young adult (AYA) cancer care. A critical barrier is the absence of a clear conceptual understanding and operational definitions of spirituality for AYAs with cancer. Purposes of this concept analysis were to: (1) clarify the concept of spirituality in the context of the AYA cancer experience and (2) generate a definition based on a review of literature examining spiritual development and the role of spirituality in AYA health and cancer treatment.

**Theoretical/ conceptual framework:** We used Roger's method of evolutionary concept analysis to identify antecedents, attributes, and the consequences of spirituality in the context of AYA cancer experience.

**Method:** We conducted a systematic search of empirical and theoretical literature across a variety of disciplines. Primary data extracted from publications included definitions and terms identified as antecedents, attributes, and consequences of spirituality. Consistent with Rodgers' method, analyses were carried out using thematic analysis and included identification of case exemplars.

**Results:** Of the 86 articles identified, 21 met our inclusion criteria (16 quantitative; two qualitative; three theoretical). Definitions of spirituality varied but shared three common features: universal experience, search for meaning, and connectedness. Analysis revealed four attributes of spirituality in AYA with cancer: meaning and purpose, connectedness, life-long universal experience, and independent of or related to religion and faith traditions. Identified antecedents included presence of spiritual resources, chronic illness, belief or wonder about a higher power, and existential questions. Identified consequences included hope, meaning, feelings of peace, and better health outcomes and illness acceptance. Based on findings, we generated a definition of spirituality in AYA with cancer.

**Conclusions**: Findings are important to development of measures and spiritual care interventions specific to AYA with cancer. However, an important limitation to address in future research is the absence of AYA first-person accounts of their own spirituality.

***Behavioral*** ***Graduate Student***

# POSTER #65

## SKIN CANCER MISINFORMATION ON INSTAGRAM: RESEARCH AND PRACTICAL IMPLICATIONS

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**Background**: Instagram isone of the most popular image-based platforms among young adults and teens. A majority (90%) of the platform's total users are aged <35 years and are at high risk for engaging in skin cancer-related behaviors. The current research state highlights the promising potentiality of Instagram as a tool to raise skin cancer awareness. However, regular use of Instagram is associated with increased utilization rates of indoor tanning services, which is associated with increased melanoma and non-melanoma cases among teens and young adults. Yet, there is a lack of research describing skin cancer misinformation on Instagram. **Objectives:** The overall goal of the study is tounderstand the landscape of skin cancer misinformation on Instagram. Our research questions were: 1) what is the prevalence and source of skin cancer misinformation on Instagram? 2) what type of media is used for misinformation posting (text, media, or video)?, and 3) what are the content categories containing misinformation (i.e., misinformation regarding skin cancer causes, treatment, and/or prevention)? **Methods:** Using CrowdTangle, we retrieved content from publicly available accounts on Instagram for the 30 days preceding May 14, 2021. A total of 592 accounts met the inclusion criteria: post content was (1) related to human skin cancer, (2) written in English language only, and (3) originated from the United States. The coding pilot and formal study processes were conducted by two dermatology residents. **Results:** Out of 522 posts reviewed, 28 (5.4%) contained misinformation related to skin cancer. More than half of the misinformation existed in the text format (n= 15/24; 62.5%). Just over one-quarter of the misinformation was presented in the posted media, such as a photo, infographics, or a video (7/24, 29.2%). Instagram accounts with non-medical backgrounds, businesses, and influencers were the main producers of misinformation. The preventive behavior theme was main content category for misinformation (n= 12/28, 42.9%) followed by treatment (n= 6/28, 21.4%) and risk factors and causes (n= 5/28; 17.9%). **Discussion:** To the best of our knowledge,this study is the first to explore the prevalence, themes, and characteristics of skin cancer misinformation on Instagram. Content that addressed prevention methods and behavior were the highest in misinformation, which aligns with findings from studies on other platforms. Future research should consider computing users' engagement with misinformation, expanding the study period, and testing possible corrective interventions to misinformation. **Conclusion:**This study responds to the U.S. Surgeon General's call to action on misinformation and skin cancer prevention. Instagram has the potential for reach out and education among teens and young adults regarding skin cancer preventive behaviors. Findings might be beneficial for dermatologists to exhibit a greater presence on Instagram and provide more expert matter corrective content on preventive behaviors.

***Behavioral*** ***Post-Doctoral/Medical Fellow***

# POSTER #66

## AWARENESS, ATTITUDES, AND PERCEPTIONS OF PHARMACISTS PRESCRIBING TOBACCO CESSATION MEDICATIONS AMONG PARTICIPANTS AT A BLACK AND MINORITY HEALTH FAIR

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**Background:**

In the United States, an estimated 68% of people who smoke report wanting to quit; however, most healthcare providers do not routinely address tobacco during clinical encounters, and few patients use evidence-based interventions when attempting to quit. To enhance patients' access to evidence-based treatment, a growing number of states (currently, n=16) now permit pharmacists to prescribe medications for cessation. To enhance our understanding of patients' perceptions of pharmacy-based assistance, this study aimed to characterize awareness and attitudes towards pharmacists prescribing tobacco cessation medications among participants of the Indiana Black Expo's Black and Minority Health Fair in Indianapolis, IN.

**Methods:**

Eligible participants who were 18 years or older and currently residing in Indiana were recruited from the Black and Minority Health Fair (July 14-16, 2022). Interested individuals were provided with a link or QR code to access and complete an electronic survey on a smart phone. Eligible participants who completed the survey received a $5 gift card. Study measures included tobacco use history, prior interactions with pharmacists, and awareness and attitudes towards pharmacists prescribing tobacco cessation medications.

**Results:**

A total of 98 surveys were completed; the majority of participants were black (83%). Approximately 13% (n=13) were current tobacco users, 21% were former tobacco users (n=21), and 65% were never users (n=64). Among current tobacco users, 54% reported a quit attempt in the last year; of these, 29% indicated they had used a cessation medication. Among all participants, 35% had been asked by a pharmacist if they used tobacco; of these, most reported being asked about tobacco use "when receiving a vaccine" (44%) followed by "when discussing health related issues" (21%). Approximately 15% of current and former tobacco users reported having been advised to quit by a pharmacist. More than three fourths of current tobacco users agreed (77%) that it would be convenient to get help with quitting at a pharmacy, 92% felt confident that they could get help from a pharmacist, and 77% felt comfortable talking with a pharmacist about quitting. Twenty-eight percent of all respondents were aware that pharmacists could prescribe medications for tobacco cessation. Of the 54% of current tobacco users who were considering quitting within the next month, 86% indicated that they intended to speak with a pharmacist about prescribing a medication for quitting.

**Conclusions:**

While previous research has found that pharmacists are interested and effective in delivering tobacco cessation services, little is known regarding public perspectives, particularly in the context of prescribing. Importantly, the current study assessed perceptions among a predominantly black population, and preliminary findings provide support for pharmacists in addressing tobacco use among this population. Furthermore, findings suggest that vaccinations might provide an opportune time for pharmacists to address tobacco use.

# POSTER #67

## ASSOCIATION BETWEEN PLASMA L-CARNITINE LEVELS AND MITOCHONDRIAL DNA COPY NUMBER

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Mitochondria are key cytoplasmic organelles of eukaryotic cells that generate adenosine triphosphate (ATP) through the electron transport chain and oxidative phosphorylation. Mitochondrial DNA (mtDNA) copy number (mtDNAcn) is considered a biomarker for both mitochondrial quantity and function as well as cellular oxidative stress level. Previous epidemiologic findings showed that weight gain, higher body mass index (BMI), smoking, and high insulinemic potential of lifestyle were associated with reduced leukocyte mtDNAcn. Carnitine, a group of compounds, plays a critical role in energy production. We quantified the associations of plasma L-carnitine levels with leukocyte mtDNAcn. We examined the association between mtDNAcn and L-carnitine (HMDB0000062) in 538 U.S. men without cancers, diabetes, or cardiovascular disease at blood collection from the Health Professionals Follow-Up Study (HPFS). We found an inverse significant association between L-carnitine (HMDB0000062) and mtDNAcn ($\rho = -0.1$, $P = 0.02$). The carnitine metabolism pathway may be associated with mitochondrial function and oxidative stress.

***Population Science/Epidemiology*** ***Graduate Student***

# POSTER #68

## THE ASSOCIATION OF SOCIODEMOGRAPHIC FACTORS ON THE TIME INTERVAL BETWEEN A SUSPECTED DIAGNOSIS OF CANCER, DIAGNOSIS, TREATING PHYSICIAN AND TREATMENT IN A COLORECTAL CANCER PATIENT POPULATION

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**Background**: Delays in colorectal cancer (CRC) diagnosis and treatment adversely affect patient outcomes; yet little is known on how socioeconomic and clinical factors impact these delays.

**Methods:** A retrospective cohort analysis utilized a randomized subset of CRC patients seen at the IU Simon Comprehensive Cancer Center between 2018-2020. Chart review identified the dates of first suspected cancer finding (SCF) based on objective predictive findings, diagnosis, first treating physician contact, and treatment initiation. Patients' demographics, setting of SCF, and modality used to discover SCF were also collected. Asymptomatic patients with SCF at routine screening were excluded. All analyses were done within a time to event framework.

**Results:** Patient characteristics (n=97): median age 61y; white 85.7%; male 55.7%; married/partnered 56.7%; never smokers 58.3%; private insurance 44.3%; employed 47.1%. SCF primarily occurred in an ambulatory setting (64.2%) after frank rectal bleeding/melena (34.0%). Median (IQR) time from SCF to diagnosis, treating physician contact, and first treatment were 20d (range 7-50.5), 28d (range 6.0-56.0), and 51.5d (range 23.5-86), respectively. Time from SCF to diagnosis was associated with SCF modality setting and diagnosis location: CT 6d (range 5-13)$p<0.01$; ED-inpatient 12d (range 6-24) $p<0.05$, ED-Inpatient: 6d(range 5-15) $p<0.05$.

Time from SCF to treatment was associated with type of biopsy used, SCF setting and diagnostic setting: forceps endoscopic biopsy 56d (range 47-70) $p<0.05$, ED-inpatient 26d (range 19-63) $p<0.05$, ED-inpatient 22d (range 12-57) $p<0.05$. Time from SCF to first appointment with treating physician was associated with tumor site: colon/large intestine 14d (range 7-34) $p<0.05$. Adjusting for confounding variables, no factors were statistically significant.
Patients with SCF through CT or Rectal bleeding/melena were 68% and 3% more likely to have stage 4 cancer compared to other modalities (FOBT, FIT, US etc).

**Conclusions:** Factors including sex, age, race, marital-status, insurance status/type, employment-status, smoking-status, SCF/diagnosis setting and modality had no impact on care intervals from SCF. Delays affecting outcomes may be occurring prior to SCF or being symptomatic may circumvent barriers to diagnosis and treatment.

***Population Science/Epidemiology*** ***Post-Doctoral/Medical Fellow***

# POSTER #69

## SOCIAL DETERMINANTS OF RACIAL DIFFERENCES IN TREATMENT AND MORTALITY IN EARLY AND ADVANCED STAGE NON-SMALL CELL LUNG CANCER IN A MIDWEST URBAN POPULATION

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**Background:** Lung cancer is the leading cause of cancer mortality worldwide. While mortality continues to decline due to advancements in early detection through low-dose CT and smoking cessation efforts, Blacks continue to have higher lung cancer incidence rates compared to Whites. Blacks have worse outcomes in non-small cell lung cancer (NSCLC) including advanced stage at diagnosis and worse survival compared to Whites. Most studies focus solely on race, missing root causes for racial disparities, including differing social, economic, and environmental factors. We analyzed exposure risk differences among Blacks and Whites with early versus advanced stage NSCLC and associated outcomes, including survival differences and stage appropriate treatment. We hypothesized racial disparities in NSCLC are mediated by negative socioeconomic factors and exposure risks disproportionately affecting Blacks, resulting in advanced stage at diagnosis, lack of receipt of stage appropriate treatment, and worse survival.

**Methods:** Individuals with newly diagnosed NSCLC, at Indiana University Simon Comprehensive Cancer Center (IUSCCC), from 1/1/2000-5/31/2015, were studied. Demographic factors included age, gender, tobacco history, race, county/zip code, and payer source. Univariate/multivariate analyses were conducted to examine the impact of identified factors on diagnosis stage, time to treatment, stage appropriate treatment, and survival.

**Results:** The cohort included 3,442 patients with newly diagnosed NSCLC, 12% (N=408) self-identified as Black. Multivariate analysis revealed those with advanced disease at diagnosis had a significantly higher odds of being male, current smokers, and uninsured. Blacks were 2.5 times as likely to be diagnosed with advanced disease compared to Whites and had worse survival. Those uninsured/underinsured were more likely to be Black compared to White. Current cigarette smokers at the time of diagnosis were more likely to be Black compared to White. Worse survival was associated with male gender, advanced stage at diagnosis, Black race, and insurance status. Racial differences in time from diagnosis to treatment or appropriate treatment for advanced disease were not observed. However, multivariate analyses showed Blacks were less likely to receive potentially curative surgery for early disease compared to Whites, regardless of insurance coverage.

**Conclusions:** This study highlights the influence of socioeconomic status on lung cancer racial disparities. Lack of adequate insurance coverage is linked to decreased access to care, less patient-physician encounters addressing known lung cancer risks, and affordability of stage-specific treatment. Our study supports a mechanism by which socioeconomic factors disproportionately impacting Blacks with NSCLC likely impact lung cancer outcomes. It suggests implicit bias may impact patient-physician practices pertaining to

potentially curative surgery for early-stage NSCLC. Our ongoing studies include analysis of the impact of environmental pollutants, neighborhood health, and measures of social deprivation on lung cancer racial disparities. Studies are needed to evaluate the impact of these factors on lung cancer racial disparities, further defining modifiable risks in screening.

***Translational/Clinical Research Faculty***

# POSTER #70

## IDENTIFYING HIGH-RISK COMPONENTS OF TRIPLE NEGATIVE BREAST CANCER BIOPSIES USING DEEP TRANSFER LEARNING

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**Background:** Triple negative breast cancer (TNBC) is the most severe breast cancer subtype with clinical features that includes poor prognosis, high metastatic potential and proneness to recur. TNBC affects roughly 15% of breast cancer patients yet still has a 5-year survival rate of only 77% in contrast to the 90% survival rate of all types of breast cancer. Due to the lack of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2) expression, TNBC patients are not sensitive to the treatment of chemotherapy. In 2019, FDA has approved immunotherapy drugs for TNBC treatment, but only 10-20% patients respond. There is an urgent need to develop effective precision medicine for TNBC patients. As a result, we advocate for the application of deep transfer learning techniques to TNBC to better understand what components, i.e. cells and tumor regions, drive these poor outcomes.

**Methods:** We used our newly developed Diagnostic Evidence Gauge of Single-cells framework to identify high risk cells and tissue regions in breast cancer. We trained a classification model based on the tumor vs. normal status of the TCGA-BRCA samples and overlaid this TCGA-BRCA tumor association onto the TNBC single cells. Patient outcome information including, disease status, survival, and BRCA subtype was learned from the TCGA-BRCA cohort and transferred to the spatial locations in the BRCA ST data. Cellular and tissue morphological image features from histopathology images was learned from the TCGA-BRCA cohort using our framework and transferred to the spatial locations in the BRCA ST data.

**Results:** We identified epithelial cells as having significantly greater tumor association than the other cell types found in the TNBC cells. We use this as a positive control since aggressive cancers that proliferate quickly should have greater numbers of malignant epithelial cells. The epithelial cells showed a large amount of variability in their tumor association indicative of both normal and malignant populations of epithelial cells. For instance, a subset of epithelial cells formed their own distinct cluster and have high tumor association. The model of tumor association learned from the TCGA patients followed the cancerous regions in the ST slide. The model of the image feature rMean_bin10 from Cheng et al. showed that this feature learned from the TCGA-BRCA data also closely followed the cancerous regions of the ST slide. The model of the epithelial cells derived from the TNBC scRNA-seq also closely followed the cancerous regions in the ST slide. These three preliminary experiments show the main uses of our deep transfer learning framework where associations with clinical, imaging, and cellular features can learned from disparate datasets and overlaid onto single cells and ST slides.

**Conclusion:** Our framework is a new approach to identify novel targets in TNBC.

***Translational/Clinical Research*** ***Faculty***

# POSTER #71

## INFLUENCE OF GENETIC ANCESTRY ON BREAST STROMAL CELLS PROVIDES BIOLOGIC BASIS FOR INCREASED INCIDENCE OF METAPLASTIC BREAST CANCER IN WOMEN OF AFRICAN DESCENT

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The biologic basis of genetic ancestry-dependent variability in disease incidence and outcome is just beginning to be explored. We recently reported enrichment of a population of ZEB1-expressing cells located adjacent to the ductal epithelial cells in the normal breast of women of African Ancestry (AA) compared to European Ancestry (EA). By establishing and characterizing cell lines corresponding to these cells and validating *in vitro* findings with tissue microarrays of healthy breast tissue from AA, EA and Latina Ancestry (LA) women, we demonstrate that these cells have the properties of fibroadipogenic/mesenchymal stromal cells that express PROCR and PDGFRa. PROCR+/ZEB1+/PDGFRa+ cells, hence renamed as PZP cells, are enriched in the normal breast tissues of AA compared to EA or LA women. *In vitro*, PZP cells trans-differentiated into adipocytes or osteocytes. In co-culture conditions, PZP:epithelial cell communication resulted in luminal epithelial cells acquiring basal/stem cell characteristics and increased expression of IL-6 suggesting the impact of this communication on the microenvironment and breast epithelial hierarchy. Consistent with this possibility, the level of phospho-STAT3, which is a downstream target of IL-6, was higher in the normal and cancerous breast tissues of AA compared to EA women. PZP cells transformed with $HRas^{G12V}$ ± SV40-T/t antigens generated metaplastic carcinoma in NSG mice suggesting that these cells could be the cell-of-origin of metaplastic breast cancers. Collectively, these results identify a stromal cell component that could influence the biology of breast cancer in AA women.

***Translational/Clinical Research*** ***Faculty***

# POSTER #72

## SEVERE SUNITINIB- AND AXITINIB-INDUCED CARDIOMYOPATHY IN A PATIENT WITH GENETIC VARIANTS ASSOCIATED WITH FAMILIAL CARDIOMYOPATHY AND REDUCED CYP3A FUNCTION

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**Background:** The anti-cancer efficacy of sunitinib and axitinib are in part mediated through inhibition of tumor-expressed vascular endothelial growth factor receptors (VEGFRs). In cardiomyocytes, VEGFR signaling is known to be cardio-protective. As a result, sunitinib and axitinib can cause cardiomyopathy in up to 30% of patients, which manifests as dyspnea and reduced left ventricular ejection fraction (LVEF).

**Methods:** A 58-year-old male who experienced cardiomyopathy during sunitinib and axitinib therapy was identified at our institutional molecular tumor board. The patient had severe dyspnea and a LVEF drop from 59% to 25% after two cycles of sunitinib. Sunitinib was discontinued, and his LVEF recovered to baseline. Two years later, he had severe dyspnea with a LVEF drop to 34% during axitinib therapy, which reversed upon axitinib discontinuation. He provided informed consent and a sample for germline whole genome sequencing (NantOmics GPS Cancer) for research.

**Results:** The patient's genome was scanned for variants associated with familial cardiomyopathy or reduced function of cytochrome P450 3A (CYP3A), the primary metabolic pathway for sunitinib and axitinib. He was heterozygous for a rare missense variant in myopalladin (*MYPN* p.Y20C; rs140148105; allele frequency: 0.002), which encodes a myocardial contractile protein and is implicated in familial dilated and hypertrophic cardiomyopathy. He was also a CYP3A5 poor metabolizer (*3/*3) and heterozygous for a very rare *CYP3A4* missense variant (p.P135L; rs1483230173; allele frequency: <0.00001) predicted to be functionally deleterious.

**Conclusion:** The *MYPN* p.Y20C variant likely contributed to the patient's drug-induced cardiomyopathy. Future work will assess the functional impact of *CYP3A4* p.P135L to determine the contributions of his *CYP3A* variants to his toxicity.

***Translational/Clinical Research*** ***Faculty***

# POSTER #73

## DUAL INHIBITION OF CDK4/6 AND PI3KMTOR BLOCKS TUMOR GROWTH IN HIGH-RISK RB-PROFICIENT OSTEOSARCOMA PATIENT-DERIVED XENOGRAFTS

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Osteosarcoma (OS), the most common type of bone cancer in children, adolescents, and young adults (AYA), relapse following or during frontline cytotoxic therapy notably in ~35% of OS patients. Currently, there are no standard second-line treatment for relapsed OS patients. Thus, there is a critical need to identify therapies targeting specific molecular signatures in OS. Hyperactivation of cyclin-dependent kinases 4 and 6 (CDK4/6) has been identified at our institution and others as a top actionable marker in OS. CDK4/6 binds to cyclin D resulting in a complex that mediates RB phosphorylation leading to cell cycle progression. While CDK4/6 inhibitors (CDK4/6i) have been well validated clinically, one drawback is that CDK4/6i induces cell cycle arrest rather than cell death. In addition, prolonged CDK4/6i therapy has been shown in other cancers to confer therapeutic resistance in RB1-proficient (RB+) tumors where compensatory pathways such as PI3K/mTOR are activated. To mitigate such CDK4/6i resistance in OS, we hypothesized that dual inhibition of CDK4/6 and PI3K pathways will promote cytotoxicity in hyperactivated CDK4/6 OS models. OS PDX models TT2-77 and HT96 (RB+, CDKN2Anull, CCND3 amplified) were treated with CDK4/6i (Palbociclib) (50 mg/kg), PI3K/mTORi (Voxtalisib) (50 mg/kg) or combination Palbociclib + Voxtalisib. In TT2-77 and HT96 PDXs treated for eight and six weeks respectively, tumor growth was significantly reduced in single-agent and combo groups compared to vehicle ($p$<0.05, two-way ANOVA). Importantly, combo was more efficacious than single agent during the last days of treatment. In addition, survival study of the PDXs, TT2-77, demonstrated an increase in the probability of survival in mice treated with combo compared with single agents. Short-term pharmacodynamic studies employing global/phospho-proteomics and kinome profiling of vehicle- versus Palbociclib-treated TT2-77 PDX showed decreased CDK4/6 pathway activation, increased activity of receptor tyrosine kinase, AXL, which is upstream of PI3K as well increased autophagy marker PIK3C3. Kinome profiling analysis of six weeks treated HT96 PDXs showed that dual inhibition of CDK4/6 & PI3K/mTOR decreased PI3K pathway activity. In addition, RB+ OS cell lines and TT2-77 xenoline were evaluated in vitro to evaluate therapy-induced mechanisms-of-action. Additive-to-synergistic cell growth inhibition at clinically relevant concentrations was evident by exposure to CDK4/6i and PI3K/mTORi. Cell cycle analysis is consistent with cytostatic effect of CDK4/6i. Apoptosis analysis on RB+ OS lines indicates that dual inhibition of CDK4/6 and PI3K/mTOR induced minimal level of apoptotic cell death. Analysis of the senescence biomarker beta-galactosidase indicated that dual inhibition of CDK4/6 and PI3K/mTOR induced senescence that was predominantly mediated by palbocicilb. These data provide evidence that Palbociclib + Voxtalisib **therapy targeting the cell cycle and decreasing PI3K pathway activity is safe, efficacious, and can decrease CDK4/6i resistance in aggressive PDX models of OS probably by induction of senescence associated cell death.**

***Translational/Clinical Research*** ***Graduate Student***

# POSTER #74

## CLINICIAN PRACTICES, KNOWLEDGE, AND ATTITUDES REGARDING PRIMARY HPV TESTING AND SELF-SAMPLING FOR CERVICAL CANCER SCREENING

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**Background:** Cervical cancer screening rates in the US are declining with notable disparities across race/ethnicity, insurance status, and sexual orientation. The inclusion of primary HPV testing without cytology in 2018 US Preventive Services Taskforce guidelines enables screening innovations, such as patient-collected samples, which may address existing barriers to screening and increase coverage of marginalized populations.

**Methods:** An online cross-sectional survey of clinicians who conduct cervical cancer screening in Indiana (n=225), followed by in-depth interviews with a subset of respondents (n=20), examined their knowledge, attitudes, beliefs and practices regarding primary HPV testing and patient self-sampling for cervical cancer screening.

**Results:** Only 3% of clinicians reported using primary HPV testing for screening-eligible patients. Although most were familiar with this method, 54% responded correctly to a knowledge question on the effectiveness of primary HPV testing, and only 50% were willing to adopt it as the preferred cervical cancer screening method for asymptomatic average-risk women ages 30-65. Most respondents (72%) believe at-home self-sampling would improve cervical cancer screening coverage, but only 50% were willing to adopt it. Interviews revealed knowledge gaps regarding primary HPV testing, and concerns about patient-collected samples including accuracy and missing out on other preventive care.

**Conclusions:** Despite guidelines and evidence on the effectiveness of primary HPV testing, almost no Indiana clinicians have adopted this screening method, and only half expressed willingness to adopt it. Although most clinicians believe patient-sampling would improve cervical cancer screening coverage, this study revealed key concerns and knowledge gaps that must be addressed to facilitate adoption.

***Translational/Clinical Research*** ***Graduate Student***

# POSTER #75

## ELUCIDATING THE ROLE OF THE AKT/EZH2 AXIS IN COLORECTAL CANCER

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Elucidating the role of the AKT/EZH2 axis in colorectal cancer
Ahmed Ghobashi and Heather O'Hagan

Colorectal cancer (CRC) develops in part through deregulation of different signaling pathways, including activation of the PI3K/AKT pathway. PI3K/AKT can be constitutively activated by mutation of proteins that regulate the pathway activity such as PTEN. PTEN (Phosphatase and tensin homolog) is a tumor suppressor protein and acts as a negative regulator for PI3K/AKT. Reactive oxygen species (ROS), generated in the tumor microenvironment, also activate PI3K/AKT. Activation of protein kinase B (AKT) mediates phosphorylation of many proteins including Enhancer of Zeste 2 (EZH2). EZH2 is a methyltransferase that trimethylates lysine 27 of histone 3 (H3K27me3) as part of polycomb repressive complex 2 (PRC2). EZH2 is proposed to act as an oncogene that induces proliferation of many tumors including CRC. However, the molecular mechanism by which EZH2 drives CRC development is not fully understood. I demonstrated that PI3K/AKT activation through PTEN knockdown (KD) or treatment with H2O2, a stable form of ROS, induced AKT to phosphorylate EZH2 at Serine 21 (pS21-EZH2). My data further demonstrated that phosphorylation of EZH2 at S21 induced EZH2 to methylate β-catenin. Aberrant WNT/β-catenin pathway activation is the initiating and promoting event in the majority of CRC. Interestingly, EZH2-mediated methylation of β-catenin induced β-catenin to interact with TCF1 (T-cell factor 1) and RNAP II (RNA polymerase II). Altogether, these findings lead me to hypothesize that AKT-mediated pS21-EZH2 induces EZH2 to methylate β-catenin to regulate β-catenin transcriptional activity, which promotes CRC proliferation. This work will further elucidate a non-histone protein target, β-catenin, of EZH2 in CRC. Current EZH2 inhibitor studies predominantly focus on H3K27me3 levels as the pharmacodynamic readout. However, the level of H3K27me3 may not be the most appropriate readout of oncogenic EZH2 activity due to EZH2's other non-histone targets. Therefore, identifying and understanding non-histone targets for EZH2 may provide promising cancer therapeutic interventions.

***Translational/Clinical Research*** ***Graduate Student***

# POSTER #76

## PROTEIN AGGREGATION PROMOTES HSF1 ACTIVITY ENHANCING CELL SURVIVAL DURING METASTATIC BREAST CANCER COLONIZATION

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Breast cancer metastasis is responsible for greater than 90% of breast cancer-related deaths among women. Metastatic colonization is rate-limiting and critical for breast cancer proliferation. Our lab has shown that HSF1 is crucial to invasion of breast cancer epithelial-to-mesenchymal transition (EMT). Utilizing an HSF1 transcriptional activity gene signature, we found that patients with high HSF1 activity have poor prognosis, suggesting HSF1 may play a role in metastasis. We have also shown increased HSF1 activity in metastatic breast tumor samples and observed a knockdown of HSF1 that reduces metastatic colonization. The mechanism by which HSF1 enables metastatic colonization is unknown. During later stages of colonization, I have found that cells with increased protein aggregation result in an increase in HSF1 activity during mammosphere formation, potentially suggesting that colonization promotes aggregation leading to HSF1 activation and cell survival.

***Translational/Clinical Research*** ***Graduate Student***

# POSTER #77

## HSF1 DOWNREGULATION OF CCL5 REDUCES CD8 + T CELL TRAFFICKING IN BREAST CANCER

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Breast cancer is the second leading cause of cancer related death in women. The presence of cytotoxic immune cells, specifically CD8+ T cells, in breast tumors is associated with better patient outcomes. Understanding the mechanisms that regulate infiltration of CD8+ T cells into breast tumors could improve the treatment and enhance patient overall survival. Utilizing a novel HSF1 activity gene signature, we observed that HSF1 activity was negatively associated with the presence of CD8+ T cells in breast cancer patients. Analysis of a cohort of breast cancer patient specimens demonstrated that patient tumors with high HSF1 activity showed lower numbers of CD8+ T cells. To test this relationship between HSF1 and CD8+ T cells, HSF1 was knocked down using shRNA in 4T1 breast cancer cells. The control and knockdown cells were injected into the mammary fat pad of immunocompetent BALB/c mice. Tumors with HSF1 knockdown had lower tumor volumes and increased CD8+ T cells infiltration. To test the functional role of HSF1 on CD8+ T cell presence, the injection of 4T1 knockdown cells into the mammary fat pad of BALB/c mice with or without CD8+ T cell depletion was examined. With CD8+ T cell depletion, the HSF1 knockdown groups had larger tumors suggesting a functional role for HSF1 to inhibit CD8+ T cell infiltration and protect the tumor from immune-mediated killing. To investigate the role of HSF1 in the regulation of cytokine secretion, the 4T1 control and HSF1 knocked down cells were subjected to a mouse cytokine screening array. The results indicated that loss of HSF1 significantly increased secretion of CCL5, which is a known chemo-attractant for CD8+ T cells. HSF1 not only regulated secretion of CCL5 but also affected mRNA levels of CCL5, suggesting a transcriptional effect on CCL5. However, HSF1 does not directly target the CCL5 gene promoter as evidenced by HSF1 ChIP-Seq, indicating HSF1 indirectly regulates CCL5 gene expression. Future directions include whether HSF1 inhibition enhances therapeutic responses to chemotherapy and immune checkpoint therapy.

***Translational/Clinical Research*** ***Graduate Student***

# POSTER #78

## TONSL IS AN IMMORTALIZING ONCOGENE OF THE CHROMOSOME 8Q24.3 AMPLICON AND NEW THERAPEUTIC TARGET IN BREAST CANCER

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Genomic aberrations that lead to immortalization of epithelial cells has been technically challenging. To overcome this technical challenge of identifying aberrantly expressed genes associated with immortalization, we utilized primary breast epithelial cells from healthy donors and their hTERT-immortalized counterparts. We identified TONSL (Tonsoku Like, DNA Repair Protein), located on chromosome 8q24.3, demonstrated that TONSL was amplified in 20% of breast cancers. Overexpression of TONSL immortalized primary breast epithelial cells and increased telomerase expression and activity. TONSL forms a complex with FACT and MMS22L1 to modulate DNA replication, repair through homologous recombination (HR). TONSL overexpression in immortalized cells increased chromatin accessibility to pro-oncogenic transcription factors including NF-kB, limited access to the tumor suppressor p53, and upregulated the HR pathway. TONSL-immortalized cells transformed with defined oncogenes generated estrogen receptor-positive adenocarcinomas in mice. Breast cancer cell lines with TONSL/chr8q24.3 amplification were sensitive to TONSL-FACT complex inhibitor CBL0137, both *in vitro* and *in vivo*. We conclude that TONSL functions as an immortalizing oncogene of the chr8q24.3 amplicon and represents a new therapeutic target in breast cancer.

***Translational/Clinical Research*** ***Graduate Student***

# POSTER #79

## TREATING HSF1-MYC COAMPLIFIED OVARIAN CANCERS WITH HDAC INHIBITORS

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Treating HSF1-MYC coamplified ovarian cancers with HDAC inhibitors

Ovarian cancer is a disease with a 5-year relative survival rate of only 49%. This low survival is in part because 80% of patients have recurrence of disease after standard platinum-based chemotherapy. This high recurrence after treatment shows the need for finding better treatments for this disease. Finding a good cancer treatment involves finding a way to target a unique biological aspect of the cancer. Our lab has found that 36% of ovarian cancer patients have copy-number amplifications of the two oncogenes Heat shock factor 1 (HSF1) and Cellular myelocytomatosis (c-MYC) which encode for transcription factors of the same name. These transcription factors are known to drive cancer through multiple mechanisms involving cell proliferation, metabolism, and survival. Because HSF1 and c-MYC have increased activity in ovarian cancers due to their amplification, finding a drug that can inhibit their activity would be an ideal treatment strategy for this specific patient population. Many drugs have indeed been developed to directly target these transcription factors; however, these drugs have had low efficacy. Besides directly targeting proteins with a drug, another way to inhibit the activity of transcription factors is to inhibit the proteins that they use to enhance their activity such as chromatin modifiers. Therefore, finding a chromatin modifier that interacts with HSF1 and c-MYC proteins would be an ideal drug target to treat coamplified ovarian cancers.

To find such a chromatin modifier, we performed a drug screen of 415 chromatin modifier inhibitors and treated coamplified ovarian cancer cell lines and non-coamplified ovarian cancer cell lines. After comparing the cell viability of the coamplified cell lines with the non-coamplified cell lines for each of the drugs, we found that the coamplified ovarian cancer cell lines were more sensitive to inhibitors of the HDACi family (inhibitors of Histone Deacetylase enzymes) than the non-coamplified ovarian cancer cell lines. To investigate why the coamplified cell lines are more sensitive to HDACi, we will use dual-reporter transcriptional assays, real time PCR, and western blot techniques to probe how HDAC inhibitors affect mRNA and protein levels of HSF1 and c-MYC as well as their direct target genes in the background of HSF1-MYC coamplified ovarian cancers.

***Translational/Clinical Research*** ***Graduate Student***

# POSTER #80

## MODIFIED ISOTONIC REGRESSION BASED DESIGN FOR PHASE I/II CLINICAL TRIALS

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Molecularly targeted agents (MTA) and immune therapy have revolutionized cancer treatment and represent the most promising new treatment in recent decades for almost any kind of cancer. Conventional phase I/II clinical trial designs often use sophisticated parametric models to characterize the joint toxicity-efficacy distributions and to conduct the trials. However, the parametric models are hard to justify in practice, and misspecification of parametric models can lead to substantially undesirable performances of phase I/II trials. Moreover, it is difficult for the physicians conducting the trials to clinically interpret the parameters of these sophisticated models, and such great learning costs impede the translation of novel statistical designs into real-world trial implementation. To solve these issues, in this presentation we propose transparent and efficient phase I/II clinical trial design, referred to as the modified isotonic regression based design (mISO). The mISO design makes no parametric assumptions on the dose-response relationship and therefore performs robustly under any clinically meaningful dose-response curves. The concise, clinically interpretable model expression and dose-finding algorithm make the proposed designs highly translational from the statistical community to the clinical community. We further extend the mISO design and develop mISO-B design to handle the delayed outcomes. Our comprehensive simulation studies show that the mISO design and mISO-B are highly efficient in optimal dose selection and patient allocation and outperforms many well-known phase I/II designs.

***Translational/Clinical Research*** ***Graduate Student***

# POSTER #81

## HUNK REGULATES IL4 IN TRIPLE NEGATIVE BREAST CANCER

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Triple-negative breast cancer (TNBC) is a type of breast cancer that does not express hormone receptors (estrogen receptor or progesterone receptor) or human epidermal growth factor receptor .Some new FDA approve as treatments for TNBC include immunotherapy targets such as immune checkpoint inhibitor (ICI). Unfortunately, inadequate anti-tumor T-cell effector function and high abundances of tumor-associated macrophages (TAMs) has limited the efficacy of ICI therapy. TAMs constituted one of the most abundant immune cell population in mammary tumors. Within the tumor TAMS can be polarized to classicallyactivatedM1-like and alternativelyactiveM2-likephenotypes. In cancer the polarization of macrophages toward a M2 phenotype is directed by cancer-cell derived factors such as pleiotropic cytokines like interleukin-4 (IL-4). New knowledge suggests that IL-4 expression in cancer cells is regulated by the signal transducer and activator of transcription 3 (STAT3) transcription factor. However, it is still undescribed what signaling pathways are responsible for mediating IL-4 in breast cancer cells. Intriguingly, we observed that Hormonally Up-Regulated Neu-Associated Kinase (HUNK), is responsible for IL-4 production in the 4T1 mammary tumor cell line. We hypothesis that HUNK regulation of IL-4 drives TAM's polarization in triple negative breast cancer cells. We engineered4T1 cells expressing HUNK and HUNK knockdown by shRNA. Our current data shown that4T1 cells expressing HUNK, have elevated levels of STAT3 phosphorylation, IL-4 production and secretion compared to 4T1 cells where HUNK has been downregulated by shRNA. Furthermore, the loss of IL-4 secretion in 4T1 HUNK knockdown cells corresponds to a reduced ability of conditioned medium from HUNK knockdown cells to induce alternative activation of macrophages. We also observed that HUNK has a significant effect on M2-likeTAMspresence in the tumor microenvironment, where tumors derived from HUNK knockdown 4T1 cells have reduced TAMs compared to control tumors. Therefore, our results proposed the identification of a HUNK signaling pathway that is responsible for pro-metastatic TAM function in TNBC. Our study will evaluate HUNK as a therapeutic target for TNBC metastasis by modulating the TAM population within the tumor microenvironment.

***Translational/Clinical Research*** ***Graduate Student***

# POSTER #82

## THERAPEUTIC TARGETING OF BET BROMODOMAIN PROTEINS IN OSTEOSARCOMA

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Osteosarcoma (OS) is an aggressive pediatric cancer with ~35% of patients developing metastasis over time. The survival rate for metastatic and relapsed OS patients is <30% and there is currently no standardized salvage therapy. Lack of efficacy is attributed to extensive genetic complexity present in OS that is partly due to moderate levels of replication stress (RS). While high levels of RS can induce cell death, moderate RS levels may cause genomic instability that contributes to progression of OS. Therefore, induction of RS to high levels, especially in genetically complex cancers like OS, could be a promising therapeutic strategy. Bromodomain and extra-terminal domain (BET) proteins (BRD2,3,4) are a family of epigenetic readers that not only regulate gene expression networks, but also regulate DNA replication and RS. BRD4 directly regulates major factors involved in DNA replication and checkpoint signaling. Thus, disruption of BRD4 function should exacerbate RS to levels that cause cell death. The objective of this study is to test the hypothesis that BET inhibition potentiates the efficacy of current salvage therapy through RS induction in aggressive OS. The effects of BET inhibitor (BETi), AZD5153, as a single agent and in combination with drugs used in salvage therapy such as topotecan were evaluated for effects on OS cell growth, PARP cleavage, and the DNA damage repair network. *In vivo* efficacy and safety studies is focused on patient-derived xenografts (PDXs) of relapsed and naive OS. TT2 xenoline, and Saos2 cell line were selected for *in vitro* experiments. Combination index and Bliss independence analyses demonstrated additive to synergistic cell growth inhibition upon treatment with clinically relevant concentrations of AZD5153+topotecan. Moreover, treatment with ARV825, a cereblon-based PROTAC that degrades all BET proteins, resulted in similar growth inhibitory effect. Significant increase in PARP cleavage was observed following AZD5153+topotecan treatment compared to single agent, indicating enhancement of apoptosis. In addition, Western blot and RNA-seq analyses demonstrated that BETi induces its effect, at least partly, through transcriptional dysregulation and increased DNA damage and RS. Dose-finding studies of AZD5153 and topotecan in OS PDXs that harbor replication stress signatures (TT2 and PDX96) indicated that daily doses of 1.25 or 2.5 mg/kg AZD5153 and 2.5 mg/kg topotecan were well tolerated and effective in partially suppressing tumor growth compared to vehicle ($p<0.05$, Two-way ANOVA; Holm-Sidak). *In vivo* combination treatments of BETi+topotecan showed that AZD5153 could potentiate the anti-cancer effect of topotecan in TT2 PDX model and increase the probability of survival in mice. These data collectively suggest that BET inhibition alongside salvage therapy holds promise as a novel treatment strategy for inducing RS-mediated cell death in aggressive OS.

***Translational/Clinical Research*** ***Graduate Student***

# POSTER #83

## THE HDAC INHIBITOR ROMIDEPSIN BLOCKS GROWTH OF METASTATIC OSTEOSARCOMA BY INDUCING CELL CYCLE ARREST

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Osteosarcoma is the most common primary malignant bone tumor and predominately affects adolescents, young adults, and canines. The five-year survival rate of human patients with detectable pulmonary metastases is only 30% and most dogs succumb to metastatic disease within a year, indicating that standard therapy (MAP: methotrexate, adriamycin, cisplatin) is not effective in these patient groups. It is therefore necessary to identify novel therapies for osteosarcoma that target the progression of pulmonary metastases. Our lab previously screened 114 FDA-approved anti-cancer drugs to identify agents that decrease the growth of 3D spheroids (sarcospheres) generated from highly metastatic osteosarcoma cell lines. Sarcospheres more closely mimic pulmonary metastases compared with a cell monolayer. The top hits from the initial screen included both histone deacetylase inhibitors (HDACi's) that were tested. In follow-up experiments with and without MAP, romidepsin was the most potent and safest of the five FDA-approved HDACi's and the seven that are in clinical trials. Our goal was therefore to further evaluate romidepsin as a potential therapy for metastatic osteosarcoma. Romidepsin decreased viability of sarcospheres generated from metastatic osteosarcoma cell lines with IC50s of 3nM-30nM and was additive to synergistic with MAP. Romidepsin also decreased viability of human and canine patient-derived cells with IC50s comparable to the human cell lines – 11 and 77nM respectively. Over 48 hours, romidepsin prevents sarcosphere growth at all drug doses, however, cytotoxicity is only present with the highest dose, suggesting that romidepsin causes cell cycle arrest. Flow cytometry cell cycle analysis shows that romidepsin blocks the cell cycle at the G1/S phase. The protein p21, which is known to mediate the G1/S transition, was found to be increased with romidepsin treatment. In an *in vivo* tail vein injection model of osteosarcoma lung metastases, the two highest doses of romidepsin prevented bodyweight loss during the experiment. Histology analysis of lung metastases at the endpoint of the study shows a trend toward the reduction of metastatic area and the number of metastases with high doses of romidepsin. This study identified romidepsin as a promising therapeutic to target the progression of micrometastases in osteosarcoma. These *in vitro* sarcosphere results show that romidepsin decreases sarcosphere viability and causes a cell cycle arrest and increases levels of p21. Further *in vivo* experiments will evaluate romidepsin in combination with standard of care MAP chemotherapy to determine how this drug combination affects survival and metastatic burden. In addition, future *in vitro* studies will further elucidate the mechanism of action of romidepsin, which will lead us to explore other therapies that can be used most effectively in combination with romidepsin.

***Translational/Clinical Research*** ***Graduate Student***

# POSTER #84

## THE MULTIDISCIPLINARY ONCOLOGY VITALITY AND EXERCISE PROGRAM (MOVE): PATIENT NEEDS ASSESSMENT AND PROGRAM DESIGN

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**Introduction** Physical activity (PA) is beneficial to all biological and physiological systems. PA also improves cognitive function, QoL, fatigue, depression, anxiety, and emotional well-being. Despite the positive effects of PA, cancer survivors do not meet the ACSM recommended PA guidelines of 150 min/wk of aerobic exercise and 2d/wk of strength training to illicit positive health outcomes. Cancer, its treatment, and side effects are complex. A comprehensive, multi-disciplinary care approach allows patients to be monitored for safety, progression, modifications, behavior change, and tailored resources for their multi-faceted needs.

**Purpose** To describe patient needs and program design of the Multidisciplinary Oncology Vitality and Exercise (MOVE) program delivered to cancer patients in one cancer care center (CCC).

**Methods:** Cancer patients (n=374; F=216, 57.8%), completed a survey about their experiences at the CCC. Items included PA participation in the last seven days, barriers, and preferences.

**Results:** Most (77%) did not meet PA guidelines citing fatigue (22.9%), lack of motivation (18.4%), and time constraints (16.1%) as the primary barriers to PA. Most (82.8%) received no PA information about resources from the oncology team or cancer center and 61.8% reported their oncologist did not mention PA for survivor treatment.

**Discussion:** A patient-centered care team designed the MOVE program to integrate functional assessment, rehabilitation, PA, and comprehensive supportive care for cancer patients. It establishes a clinical infrastructure that will improve patient PA, physical function, and QoL.

**Current Progress and Future Direction:** After a cancer diagnosis, an initial assessment is completed by a Physical Therapist (PT) Cancer Specialist and the patient completes several questionnaires assessing symptom burden including a PROMIS-29. Next the patient receives a personalized support prescription including one of the following: 1) continued PT 2) referral to a PA program: supervised, unsupervised, home-based, community-based or 3) referral to other resources to optimize care. To date, 55 patients have completed a PT MOVE program assessment. Future work will describe the MOVE program's progress toward transformative research, impactful public health policy, education, and personalized care.

***Translational/Clinical Research*** ***Graduate Student***

# POSTER #85

## IDENTIFICATION OF MCAK INHIBITORS THAT INDUCE ANEUPLOIDY IN TRIPLE NEGATIVE BREAST CANCER MODELS

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The standard of care for triple negative breast cancer (TNBC), the most lethal breast cancer subtype, is chemotherapy using a combination of microtubule poisons and DNA damaging agents. Microtubule poisons, like paclitaxel, have been proposed to induce lethal levels of aneuploidy in tumor cells. While these drugs are initially effective in treating cancer, dose-limiting peripheral neuropathies are common, and patients often relapse with drug resistant tumors. Developing agents against targets that limit aneuploidy may be a valuable approach for therapeutic development. One potential target is the microtubule depolymerizing kinesin, MCAK, which limits aneuploidy by regulating microtubule dynamics during mitosis. Analysis of MCAK expression levels in the TCGA and GSE47651 breast tumor databases showed MCAK to be upregulated across all breast cancer subtypes, with the highest levels in TNBC. Additionally, high MCAK expression was associated with reduced overall survival and distant metastasis-free survival, indicating that MCAK may be able to serve as a biomarker of disease severity. Knockdown of MCAK in tumor-derived cell lines caused a 2 to 5-fold reduction in the $IC_{50}$ for paclitaxel, but there was no change in normal diploid lines, indicating that MCAK loss may have cancer-specific effects. Treatment of cells with paclitaxel or knockdown of MCAK both caused an increase in aneuploidy, but combination treatments did not have an additive effect, suggesting that another mechanism is likely responsible for the increase in taxane sensitivity. Interestingly, MCAK knockdown also induced aneuploidy in a taxane resistant breast cancer line. To identify potential MCAK therapeutics, we developed two screens using FRET and image-based assays and identified three candidate inhibitors. Similar to our knockdown studies, these drugs increased aneuploidy in breast cancer cells, regardless of taxane-resistance. These inhibitors also caused a potent reduction in colony formation assays in both taxane-sensitive and resistant cells. The two most potent inhibitors, B4 and C4, also led to an approximate three-to-five-fold reduction in the $IC_{50}$ of paclitaxel in tumor-derived lines. Collectively our work will expand the field of precision medicine to include aneugenic drugs, while giving treatment options to breast cancer patients with relapsed or drug-resistant disease.

***Translational/Clinical Research*** ***Graduate Student***

# POSTER #86

## REGULATION OF INITIATION AND ESTABLISHMENT OF OVARIAN CANCER METASTASIS BY METASTASIS INITIATING CELLS

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Ovarian cancer (OC) is the deadliest gynecologic malignancy with a dismal prognosis worldwide. A key reason for this is that most patients already have extensive metastasis at the time of diagnosis. Therefore, it is metastatic disease that is being treated in the clinic. However, metastasis remains one of the least understood aspects of cancer biology and a better understanding of the mechanisms involved will be helpful in devising more effective therapies. Transcoelomic metastasis is prevalent in ovarian cancer, which involves exfoliation of cancer cells in the primary tumor, floatation and spreading in the peritoneal fluid, followed by attachment, and colonization of the peritoneal organs. The most common site being the omentum. Recent research has indicated that a small percentage of OC cells (metastasis-initiating cells - MICs) are able to survive at the metastatic site and establish metastases. However, the characteristics of MICs, their interactions with surroundings and how they colonize metastatic sites remains to be explored. We used an organotypic 3D culture model of the surface layers of the omentum to mimic early stage of metastatic colonization and a patient derived xenograft model to represent advanced metastasis. Single cell transcriptomics was performed to identify the heterogeneity of the patient derived OC cells and the changes in their subpopulation profiles during early and advanced metastasis. Extensive bioinformatic analysis of the single-cell transcriptomics helped identify and characterize the MICs. We identified two distinctive populations of OC cells with MIC-like phenotype and have determined the potentially targetable differentiation trajectory between the MICs and other cancer cells populating the established metastases. Elimination of MICs or interruption of their differentiation trajectory paths that help establish the metastases may be a novel therapeutic opportunity leading to improved outcomes for the OC patients who are diagnosed with metastasis.

***Translational/Clinical Research*** ***Graduate Student***

# POSTER #87

## OSTEOSARCOMA PATIENT DECISION AID FOR SURGERY IN THE LOWER LIMB: ALPHA TEST RESULTS WITH STAKEHOLDERS

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Background: Osteosarcoma is a rare bone cancer with about 1,000 cases annually in the United States. Most tumors present in around the knee joint; surgical options include amputation, rotationplasty, and limb salvage surgery. Families and patients often struggle to find clear information to understand risk, benefits, and long-term outcomes of the options.The International Patient Decision Aid Standards guided patients, parents, and providers in the development of an online decision aid for future families.This IRB-approved study tested the first version of this decision aid.

Methods: Asurvey to review the online decision aid was disseminated during the Osteosarcoma Conference in June 2022 and remained open for eight weeks. Participants were asked to comment on what they liked, didn't like, and what recommendations they would make for each section of the website, and received a gift card for their time.

Results: Participants (n=33) were survivors (15), parents (11), providers (5), and researchers (2). Content analysis was performed by all three coders who identified fourfocus areas in their comments:content, structure, visuals, and accessibility. Participants felt the content was clear, thorough, and relatable. They liked the testimonials, support links, and long-term outcomes, but disliked a noted bias: they felt it needed more evidence and citations. Visually they liked the images, PDFs, videos, and the layout of the site, but disliked the lack of diversity and age range of patients featured across the tool. Participants also wanted more images to break up the text-heavy page layout.They felt the structure was generally well organized and liked the bullet points, checklists, and color blocking, but felt it could be even more clearly laid out. For accessibility, it was well-written and easy to both read and understand, but some felt it could be overwhelming and was very text heavy with inconsistent use of text. Recommendations include more visuals, specific content additions, larger font, links to scientific studies, a glossary, increased accessibility (e.g., captions for videos), and a balance in the overall tone of the site between offering realistic expectations and hope.

Conclusions: Revisions will be made in consultation with a medical advisory team. The second iteration of the decision aid will be beta tested with end users from the general public.

Next Steps: This is the first decision aid for this patient population, resulting in comprehensive information in one location. Following beta testing, it will be revised accordingly, and pilot tested with patients and parents making the decision.The final version will be freely available to surgeons and patients, with distribution in patient support groups for this population.

***Translational/Clinical Research*** ***Graduate Student***

# POSTER #88

## PARACRINE INTERACTIONS WITH ADJACENT CANCER ASSOCIATED FIBROBLASTS IMPART CHEMORESISTANCE TO OVARIAN CANCER CELLS

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Ovarian cancer is the most lethal gynecologic cancer and the fifth leading cause of cancer-related deaths among women in the US. This high death rate is mainly due to extensive metastasis and a high recurrence rate. The standard of care involves debulking surgery and Carbo-Taxol chemotherapy. While most patients respond well initially, some cancer stem cells survive and cause ovarian cancer relapse. Cancer-associated fibroblasts (CAFs) are key components of the tumor microenvironment (TME) that have been suggested to play important roles in regulating tumorigenesis. Moreover, the residual tumors following neoadjuvant chemotherapy are typically fibrotic, indicating the potential role of CAFs in providing a niche for surviving cancer cells.

Like cancer cells, CAFs are heterogenous and our research has shown a subpopulation of CAFs secrete WNT5A to regulate cancer cells and enrich the cancer stem cell population. My analysis of multiple publicly available datasets revealed that CAFs have significantly higher WNT5A expression than cancer cells, further confirming the role of CAF-derived WNT5A in clinical samples. Deconvolution of TCGA ovarian cancer data further revealed that the percentage of CAFs in the TME increases with the cancer stage. Deconvolution analysis of the Australian Ovarian Cancer Study dataset containing transcriptomic information of chemo-resistant and chemo-sensitive patient tumors showed significant CAF enrichment in chemoresistant tumors. TUNEL and immunofluorescent staining were done in frozen sections of chemo-naïve ovarian cancer patient tumors and compared to tumors from patients who had received prior chemotherapy. While chemo naive tumors had minimal apoptotic cells, chemotherapy-induced apoptosis in cancer cells was further away from CAFs. Interestingly, the cancer cells adjacent to CAFs were spared.

Our previous research had shown that WNT5A triggers cancer stem cell enrichment in two ways. It stimulates ovarian cancer stem cell self-renewal by promoting symmetric division and increases the dedifferentiation of subpopulations of bulk ovarian cancer cells. To identify and characterize the subpopulations of CAFs that are capable of inducing stemness and chemoresistance as well as the subpopulation of ovarian cancer cells that are responsive to these signals, we applied a single-cell RNA sequencing (scRNA-seq) approach. Heterotypic 3D cocultures of patient-derived ovarian cancer cells and CAFs were thus analyzed. Published datasets further allowed us to validate our findings in patient samples. We characterized the pathways activated in CAF subpopulations with high WNT5A expression, and found upstream targets of WNT5A expression and secretion.

We also found cancer cell subpopulations, that respond to CAF signals and become cancer stem cells, using trajectory inference and ligand-receptor network analysis. Our research uncovered the heterogeneity of CAFs in ovarian cancer TME and demonstrated the role of certain subpopulations that can serve as a cancer stem cell niche, giving rise to disease relapse. We also uncovered how OC cells modulate CAFs in TME by secreting ligands and increasing $WNT5A^{high}$ CAFs. My long-term goal is to determine the molecular mechanism of the CAF-cancer stem cell crosstalk and specifically target this communication to prevent ovarian cancer recurrence and improve patient outcomes.

***Translational/Clinical Research*** ***Graduate Student***

# POSTER #89

## A QUALITATIVE STUDY OF A NEW METRIC FOR ESTIMATING THE RISK OF EARLY-ONSET COLORECTAL CANCER IN MALE VETERANS

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**Background:** Identifying patients who benefit from screening prior to ages 45 or 50 through risk-prediction models may improve the uptake and effectiveness of colon cancer (CRC) screening. “Colon Age” is a way to estimate a person’s risk for CRC in Veterans younger than age 50. The purpose of this study is to determine what patients and primary care providers think about the “Colon Age” concept in terms of its comprehensibility, acceptability, and utility for decision-making about CRC screening.

**Methods:** Veteran patients < 50 years old andprimary care providers (PCPs) were recruited from the Roudebush VAMC’s outpatient clinics. Semi-structured, qualitative interviews were conducted one-on-one and included Likert-scale and open-ended questions. The estimate of colon age is based on relating a person’s individual features to the 5-year age group in the U.S. population of men with the closest risk of CRC based on Surveillance, Epidemiology, and End Result cancer incidence data.

**Results:** 19 patients and 8 PCPs were recruited. The majority of patients (68%) had a colon age below that of their biological age, while 2 of 19 (11%) had a higher colon age. PCPs identified the tool’s potential to promote screening uptake, facilitate discussion between patients and PCPs, and adhere to current practices as facilitators. Identified barriers among PCPs and patients included questions about tool accuracy and validation, patient reluctance to receive any form of screening, and limited perceived utility for patients between the ages 45-49.

**Conclusions and Potential Impact:** Using the concept of colon age to express individual patient risk for early-onset CRC was well-received among almost all veteran patients and physicians interviewed.Colon-age adapted screening tools should be incorporated into the electronic health record reminders to facilitate decision-making and screening uptake.Colon-age based personalized screening has the potential to improve cancer detection by providing individualized recommendations.

***Translational/Clinical Research*** ***Medical Student***

# POSTER #90

## FACTORS INFLUENCING DISEASE RECURRENCE AFTER PRIMARY R0 RESECTION OF MASAOKA STAGE I AND II THYMOMA

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**Background:** The available research has demonstrated that the best treatment for early thymoma is surgical resection, with the goal of an R0 (complete) resection and an associated cure rate of 90-95%. The standard approach is a median sternotomy with en bloc resection of the thymoma, though minimally invasive approaches (video-assisted and robotic-assisted thorascopic surgery; VATS/RATS, respectively) have recently gained popularity. These techniques remain controversial as tumor spillage into the pleural space is difficult to control and increased tumor manipulation during minimally invasive surgery may increase risk of drop metastases.

**Purpose:** This study analyzes factors influencing disease recurrence after primary R0 resection of Stage I/II thymoma, with a specific focus on the effect of surgical approach on probability of disease recurrence.

**Methods:** A database of 1023 thymic neoplasm patients seen at IU was established. From this database, 109 patients with stage I/II thymoma and primary R0 surgical resection were identified. Cerner records were reviewed retrospectively, and the following data were collected: (1) WHO histologic type, (2) biopsy prior to surgery (yes/no), (3) surgical approach, (4) recurrence status/location, and (5) location of surgery (IU or outside institution).

**Results:** Of the 109 patients, 30 had recurrence/progression after surgery. 25 VATS/RATS surgeries were performed, with 7 cases of recurrence (28%), and 81 open surgeries were performed, with 20 cases of recurrence (24%); 3 patients with recurrence did not have sufficient records to determine surgical approach. For the VATS/RATS patients with recurrence, 100% had pleural recurrence ipsilateral to the surgical approach. 57 surgeries were performed at IU, with 2 patients having recurrence (96.5% cure rate at IU), while 52 were performed outside IU, with 28 patients having recurrence. Biopsy prior to open sternotomy was associated with a 38.9% recurrence rate, whereas open sternotomy without biopsy had a 17.1% recurrence rate. Lastly, WHO types B1, B2, and the mixed B2/B3 type were associated with increased risk of recurrence.

**Conclusions:** Surgical approach, performance of biopsy prior to surgery, WHO histologic type, and surgical institution, are all factors that may influence recurrence probability in stage I/II thymomas. In addition, this study demonstrates that pleural recurrences after VATS/RATS tend to occur ipsilateral to the surgical approach. This finding further supports the concern that there could be greater risk of pleural recurrence with minimally invasive surgery as a result of increased tumor manipulation; a similar mechanism is theorized to be responsible for increased recurrence risk with biopsy. Lastly, the low recurrence rates at IU suggest thymoma resection should be performed at centers with extensive thymoma experience.

Limitations: The cohort analyzed here had a higher recurrence rate than reported elsewhere for stage I/II thymoma. This is likely a selection bias based on IU's experience with advanced thymoma.

***Translational/Clinical Research*** ***Medical Student***

# POSTER #91

## BARRIERS AND FACILITATORS TO A MEDITERRANEAN DIET INTERVENTION DURING CHEMOTHERAPY TREATMENT: A QUALITATIVE ANALYSIS

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Patients undergoing chemotherapy for cancer tend to experience many side effects including fatigue, nausea, vomiting, loss of appetite, and mouth sores, which reduce quality of life. The stress of a cancer diagnosis and its treatments can be compounded by malnutrition, with malnutrition affecting more than half of patients with cancer. Thus, the overall goal of this project was to identify barriers and facilitators to adhering to a nutritional intervention that has potential to enhance patients' health and quality of life during treatment. This project was a subcomponent of a larger study, the Diet and Nutrition in Cancer Trial (DANICA), which tested the effects of an 8-week Mediterranean Diet (MedDiet) intervention on cancer-related fatigue among patients actively undergoing chemotherapy. The participants received weekly food deliveries (12 frozen meals/week, e.g., falafel plate, baked ziti) plus groceries (e.g., vegetable juice, whole wheat pasta) for 4 weeks. Participants were also provided educational materials with information about the MedDiet, along with a cookbook with MedDiet recipes. Participants had a one-on-one session with a nutrition scientist at week 3 to discuss behavior change techniques and set goals. The control group was neither encouraged nor discouraged to change their dietary habits and received all the intervention materials at the end of their time in the study. Following completion of the 8-week intervention, participants completed semi-structured exit interviews to understand their experiences with the MedDiet intervention during active chemotherapy treatment. Qualitative analysis was conducted with 29 interview transcripts using MAXQDA software. Interviews were independently coded by 2 reviewers, using emergent themes as codes. Participants were 51.0±x15.1 years old and 93.1% had breast cancer. As facilitators to MedDiet adherence, the educational materials were the highest reported (86%), with the convenience of having things delivered (52%) being the next common. Several patients offered that changing their diet fulfilled a welcome sense of control and empowerment. The most reported barriers to MedDiet adherence were that the provided food was unappetizing (38%), and that participants' food preferences did not align with the MedDiet (33%). Ultimately, this project will address the gap in understanding the patient experience in nutritional interventions during active chemotherapy treatment. This data can be used in future research to optimize dietary programs during chemotherapy treatment.

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***Translational/Clinical Research*** ***Medical Student***

# POSTER #92

## HISTOLOGIC DIVERSITY OF THYMIC EPITHELIAL TUMORS IN PATIENTS WITH MYASTHENIA GRAVIS

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**Background:** Thymic epithelial tumors (TETs) include thymic carcinomas and thymomas, the latter of which can be further categorized by the World Health Organization (WHO) histologic classification based on the morphology of epithelial cells and the ratio of lymphocyte to epithelial cells (WHO types A, AB, B1, B2, and B3). TETs are rare malignancies with an incidence of 0.15 per 100,000 person-years in the United States. While their etiologies remain unknown, these tumors are associated with distinctly high rates of autoimmune disorders and paraneoplastic syndromes. The most common comorbid autoimmune disorder is myasthenia gravis (MG), affecting approximately 30% of patients with thymoma; thus, evaluating the risk of MG in patients with TETs of various histologies is important clinically. For the present retrospective study, we created a database of patients with TETs and examined prevalence of each histologic subtype in patients with MG.

**Methods:** Drs. Patrick Loehrer, Kenneth Kesler, and colleagues have collaborated at the Indiana University Simon Cancer Center to care for over 1000 patients with TETs. The electronic health records of these patients were accessed via Cerner and used to input demographic, diagnostic, and histologic data into a REDCap database. The TETs were further categorized by WHO classification, and heterogenous tumors were categorized by their most aggressive histologic type (i.e. mixed type B2 and B3 categorized as B3).

**Results:** Of 1023 total patients in the REDCap database, 626 were found to have sufficient documented information regarding TET diagnosis and histology as well as the presence or absence of MG (thymoma – 468; thymic carcinoma – 158). 112 of these patients carried diagnoses of both MG and a TET confirmed by pathology report (thymoma – 110; thymic carcinoma – 2). 77 (68.75%) patients were diagnosed with MG prior to TET, while 30 (26.79%) were diagnosed with MG after TET ($p < 0.0001$). The greatest prevalence of WHO histologic type in patients with thymoma and MG was Type B3 (36, 32.14%), followed by Type B2 (33, 29.46%), Type B1 (19, 16.96%), Type A (7, 6.25%), and Type AB (7, 6.25%) ($X^2 = 37.41$, $p < 0.0001$). Notably, only 2 of 158 (1.27%) total patients with TC had comorbid MG in contrast to 110 of 468 (23.50%) with thymoma and MG; this suggests a uniquely favorable microenvironment of thymoma in patients with MG.

**Conclusions:** A distinct link exists between myasthenia gravis and thymoma, particularly those of more aggressive WHO histologic types (Type B3 and Type B2). Future work will aim to determine whether histologic classification has a predictive value for tumor prognosis in patients with and without MG. Furthermore, patterns of gene expression associated with thymoma in patients with and without MG may elucidate the etiologic mechanisms for the development of this autoimmune disorder.

***Translational/Clinical Research*** ***Medical Student***

# POSTER #93

## TRENDS IN METASTASES AMONG PATIENTS WITH MASAOKA-KOGA STAGE IV THYMIC EPITHELIAL TUMORS

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Background: Thymic epithelial tumors (TET's), including thymomas (5 histological subtypes) and thymic carcinomas, are rare tumors with an estimated incidence of 0.15 per 100,000 person-years in the United States. While their etiologies remain largely unknown, some are associated with uniquely high rates of paraneoplastic syndromes and an elevated risk of secondary malignancies. And, though thymomas were once thought to be benign tumors, it is now well-documented that all TET's can metastasize. The gold-standard in TET staging, the Masaoka-Koga system, defines metastatic disease as Stage IV, further specifying pleural/pericardial metastases as Stage IVa and lymphatic/hematogenous metastases as IVb. Unfortunately, little is known about patient prognosis as it relates to metastasis location. Here, we assemble and analyze one of the largest single-institution databases of TETs in the world and seek to examine trends in their metastasis.

Methods: Files of 1023 TET patients seen at Indiana University Hospital were accessed via Cerner, after which a standardized information list including demographics, diagnostics, tumor histology, treatments used, disease course, and patient outcome at last follow-up was extracted and input into a RedCap database.

Results: From 1023 patient files we identified 467 thymomas and 159 thymic carcinomas. 267 of those thymomas (57%) and 122 of those thymic carcinomas (77%) were confirmed to have Stage IV disease at diagnosis or at some point during their disease course. The most common site of metastasis overall for both thymoma and thymic carcinoma was the pleura (260patients), whereas the most common extra-thoracic site of metastasis for both tumors was the liver (66 patients). However, patterns of spread differ between the tumors, with thymomas metastasizing to the pleuraand lung parenchyma more frequently and thymic carcinomas metastasizing to the bone, cervical lymph nodes, and kidney more frequently. Also notable is that 158 of our Stage IV patients (41%) presented with extra-thoracic metastases, including 29 of them whopresentedas such without any intrathoracic metastases.

Potential Impact: These data altogether confirm that patterns of metastasis vary with tumor histology and further suggest that disease spread outside the thorax occurs more commonly than previously reported. Later, routine screening for this cancer may benefit from abdominal/pelvic scans. Future directions for this work may include elucidating the correlation between patient prognosis and patterns of metastasis in this cancer.

***Translational/Clinical Research*** ***Medical Student***

# POSTER #94

## THYMOMA AND PURE RED CELL APLASIA: A SINGLE INSTITUTION EXPERIENCE

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**Background:**

Thymoma is an uncommon malignancy often associated with paraneoplastic syndromes including pure red cell aplasia (PRCA), occurring in up to 5% of patients with thymoma. Considering the rarity of thymoma-associated PRCA, optimal treatment regimens and impact on thymoma disease course have yet to be established.

**Methods:**

A retrospective chart review was conducted for patients seen at the IUSCCC with thymoma and PRCA to extract and synthesize data on patient demographics, thymoma histology, hemoglobin levels, timeline of diagnoses, treatment regimens, and outcomes.

**Results:**

From 1993 to 2022, 10 patients (M:F = 4:6) with thymoma and PRCA were identified. Median follow-up duration was 75.0 months (range 32-202). Median age at thymoma diagnosis was 53.5 (range 40-80). Median age at PRCA diagnosis was 64.0 (range 40-80). Mean hemoglobin at PRCA diagnosis was 6.6. WHO classifications of thymoma included 10.0% A, 40.0% B1, 10.0% B2, 10.0% B2/B3, 30.0% not otherwise specified. Masoaka stages of thymoma included 20.0% stage I, 10.0% stage II, 10.0% stage III, 30.0% stage IVA, 30.0% stage IVB. Patients initially presented with thymoma (n=7), PRCA (n=1), or concurrent thymoma and PRCA (n=2). For those initially presenting with thymoma, PRCA was subsequently diagnosed after a median 19.5 months (range 9-144). Of these 7 patients 3 experienced a relapse or progression of their thymoma at time of PRCA diagnosis, 3/7 had stable disease (1-insuffiecient data). Additional paraneoplastic syndromes were seen in 6 patients (hypogammglobulinemia-2; pure white cell aplasia-1; myasthenia gravis-1; autoimmune neutropenia-1; colitis -1). Other diminished immunologic parameters observed were: immunoglobin levels (n=3); CD4 counts (n=2); CD4:CD8 ratio (n=4); and total B cell (n=6). The primary outcome of the study was transfusion dependence, defined as requiring ≥1 PRBC transfusion per month for ≥3 months. Six achieved transfusion independence, while 3 remained transfusion dependent despite therapy (1 LTFU). Both patients with concurrent thymoma and PRCA underwent thymectomy, but only one achieved transfusion independence with additional adjuvant immunosuppressive therapies. Median duration of transfusion independence was 18.5 months (range 4-204). Median survival after bone marrow biopsy diagnosis of PRCA was 50.0 months (range 9-202).

**Conclusions:**

The impact of PRCA on thymoma disease course is variable. Most patients will recover with immunosuppressive therapy, but approximately 30.0% patients remained transfusion dependent. Our

experience does not support thymectomy alone as primary treatment of PRCA. More research is needed to evaluate the mechanism of developing PRCA in thymoma and to define the optimal therapeutic approach.

***Translational/Clinical Research*** ***Medical Student***

# POSTER #95

## OUTCOMES OF ARTERIAL AND CAVAL RESECTION DURING POST-CHEMOTHERAPY RETROPERITONEAL LYMPH NODE DISSECTION IN METASTATIC TESTICULAR CANCER

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**Background/Objective:**

In the United States, testicular cancer is the most common solid tumor in men aged 15 to 34. Fortunately, testicular cancer has a cure rate greater than 90% and a 97% five-year survival rate. For the men not cured, a relapse to the retroperitoneum (RP) is most common. Of the patients with RP metastases, a minimal number may require post-chemotherapy retroperitoneal lymph node dissection (PC-RPLND) with resection of the aorta, external iliac, or inferior vena cava (IVC). We hypothesized that this procedure would yield reasonable cure rates with acceptable levels of postoperative complications to warrant the indication for surgery.

**Methods:**

Between 2000 and 2020, 2,054 patients with metastatic testicular cancer underwent a PC-RPLND; of those men, 39 also underwent an aortic, external iliac, and/or IVC resection. For the men with a PC-RPLND and vascular resection, demographic, clinical, pathologic, and operative information were reviewed. Next, a Kaplan-Meier curve was created to determine overall survival.

**Results:**
In this retrospective cohort study of 39 patients, PC-RPLND and vascular resection occurred at a median age of 40. The median follow-up of the cohort was 9 months. The median pre-operative mass size was 9 cm and 19 cm in the RP and pelvis, respectively. At PC-RPLND, 54%, 13%, 18%, and 15% of patients demonstrated cancer, teratoma, teratoma and cancer, and necrosis, respectively. Following PC-RPLND and vascular resection, 22 (56%) patients recurred. The median (IQR) time to relapse was 4.2 (2.5 – 8.2) months. The most common location for recurrence was to the lung, followed by the RP and liver. In total, 17 (44%) patients died of disease. The median overall survival was 14.8 months, and at two years, overall survival was 45%.

**Conclusion:**
With an overall survival rate of 45% at two years in this heavily pretreated patient population, PC-RPLND with resection of the aorta, external iliac, and/or IVC is reasonable in very select cases.

***Translational/Clinical Research*** ***Medical Student***

# POSTER #96

## EVALUATION OF CHEMOTHERAPEUTIC OUTCOMES FOR THYMIC CARCINOMA PATIENTS

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### Evaluation of Chemotherapeutic Outcomes for Thymic Carcinoma Patients

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**Background and Hypothesis:**

Thymic Epithelial Tumors are uncommon tumors of the anterior mediastinum composed of thymomas and thymic carcinomas (TC). TC's are known to have worse disease outcomes and lower rates of survival in comparison to thymomas and are suspected to have lower response rates to chemotherapy as well. As these tumors are rare, little data exists assessing the true efficacy of chemotherapeutic regimens for TC patients. Due to this lack of data, and that the Indiana University Health Simon Cancer Center treats a high percentage of TET patients, a database of these patients covering a variety different disease characteristics and treatments has been established.

**Methods:**

In this project, a collection of patients seen by Dr. Patrick Loehrer and/or Dr. Kenneth Kesler was acquired, and a database was created using these patients in RedCap. Once established, we evaluated patient medical records in Cerner and entered data related to disease characteristics and treatments. These patients were then analyzed accordingly to evaluate chemotherapy response rates.

**Results:**

The database yielded 159 patients shown to have TC diagnosed outside IU or at IU, and 123 instances of chemotherapy treatment for TC. Of which, the most popular treatments were PAC and Carbo/Taxol regimens. Initial data suggests that PAC generates a higher response rate (65.5%) than other therapies (Carbo/Taxol: 27.6%, PE: 58.3%, etc.). Therefore, anthracycline based regimens may be more effective at generating response rates in comparison to non-anthracycline based regimens.

**Conclusion and Potential Impact:**

This project will help elucidate the effectiveness of recommended systemic therapies for thymic carcinoma patients from one of the largest TET databases constructed. Ultimately, we hope that with clarity of the effectiveness of treatment, this can serve as a reliable reference for evidence-based medicine for the care of TC patients.

***Translational/Clinical Research*** ***Medical Student***

# POSTER #97

## SPATIAL TRANSCRIPTOMIC PROFILING OF CUTANEOUS MELANOMA PROGRESSION

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**Background and Objective:**

In 2022, an estimated 99,780 new melanomas will be diagnosed in the United States. As incidence rates continue to rise, identification of biomarkers for disease progression is urgently needed to prevent overdiagnosis and provide therapeutic targets. The purpose of this project was to determine if spatial transcriptomics can be used to identify transcript changes during melanoma progression.

**Methods:**

For this study and after specimen quality control (via DV200 values), four archival formalin fixed paraffin embedded (FFPE) human melanoma specimens were processed using the Visium Spatial Gene Expression platform. In Loupe Browser v6.0.0. (10x Genomics Inc.), K-means clustering was used as an unbiased approach in addition to manual selection of areas of melanoma adjacent to and distant from (i.e. nonadjacent) the epidermis to determine regions of interest for identification of differentially expressed genes (DEGs).

**Results:**

Expression of *PAEP* was significantly increased in a micrometastasis (~7.2 fold; $Log_2$ scale) versus the primary melanoma in one specimen. Other DEGs also distinguished the micrometastasis (*SLC16A3*, *CCND1*, *SCML4*, and *CSAG3*) from this primary melanoma (*S100A14*, *TRIM29*, *PTPRZ1*, and *BCAN*). In the other specimens, a similar pattern of differential gene expression was seen between areas of melanoma adjacent to and nonadjacent to the epidermis. In addition, K-means clustering identified a region of differential gene expression suggestive of an inflammatory cell infiltrate next to the *PAEP*-enriched micrometastasis. Initial immunohistochemical staining was also able to detect PAEP protein expression in the micrometastasis.

**Conclusions and Potential Impact:**

This study demonstrates the feasibility of using spatial transcriptomics to investigate transcriptional changes during melanoma progression. Increased *PAEP* transcripts and the immunosuppressive functions of PAEP suggest PAEP may be an important mediator of melanoma progression. The current study suggests increased *PAEP* transcript levels are associated with inflammatory cell infiltrates. Understanding mechanistic links between increased *PAEP* and inflammation during melanoma progression could provide prognostic and therapeutic insights and thus, improved care for melanoma patients.

***Translational/Clinical Research*** ***Medical Student***

# POSTER #98

## PHARMACOLOGICAL CHARACTERIZATION OF SECOND-GENERATION REF-1 INHIBITORS AND EVALUATION FOR POTENCY, TARGET INHIBITION, AND EFFICACY USING 3-DIMENSIONAL CO-CULTURE MODELS

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Targeted therapy for cancer using small molecules has progressed exponentially, but agents that can affect cancer cells rather than non-tumorigenic cells are crucial to avoid pernicious side effects. AP endonuclease-1 / Redox factor-1 (APE1/Ref-1 or Ref-1) is a multifunctional protein with DNA repair activity and redox signaling activity as major functions. The DNA repair function is indispensable for cell survival. Its role as a redox factor that stimulates the DNA binding activity of numerous transcription factors (TFs) such as HIF-1a, NFκB, STAT3, and AP-1 by reducing their critical cysteine residues, tends to be dysregulated in cancer cells. Ref-1 is overexpressed in many cancers including pancreatic ductal adenocarcinoma (PDAC) and malignant peripheral nerve sheath tumor (MPNST) making it an attractive target. We advanced a first-generation Ref-1 inhibitor (APX3330) to a phase I clinical trial in adult patients with progressing solid tumors. In this trial, we observed a 32% disease control rate, no significant toxicities, disease stabilization in six patients with four on treatment for an extended time (>250 days), identified a phase II dose, predicted PK, and target engagement. While APX3330 has successfully completed phase I trial for safety and toxicity, development of additional second-generation compounds with increased efficacy is still desirable, both for efficacy and reduction of potential off-target effects in patients. Through chemical modification of APX3330, structure-activity relationship (SAR) efforts with over 55 second-generation synthesized compounds have resulted in five compounds with improved drug-like properties, pharmacokinetics, target engagement, mouse and human S9 fraction metabolic stability, in silico ADMET properties, and increased efficacy for cell killing. Validation for direct interaction of these inhibitors with Ref-1 using ligand-based WaterLOGSY NMR measurements and EMSA are ongoing. For these second-generation compounds, we confirmed target inhibition following Ref-1 inhibition (reporter assay and qPCR), performed two physiologically relevant spheroid growth assays, and evaluated efficacy in pilot orthotopic PDAC models. Target engagement studies involving blockade of HIF-1a and NFkB activity in multiple cancer cell lines resulted in significant and dose-dependent decreases in TF-driven luciferase activity. In two 3D co-culture models (interstitial T-MOC and 3D spheroid), second-generation Ref-1 redox analogs suppressed tumor survival significantly while sparing cells from the tumor microenvironment. These findings are being confirmed *in vivo* with a panel of patient-derived orthotopic xenografts. The PDX panel will also allow us to identify responders and non-responders as well as mechanisms of resistance. This study aims to provide invaluable information regarding the new analogs for advancement to *in vivo* and eventual IND enabling studies and to demonstrate the effectiveness of new Ref-1 analogs in sophisticated *in vivo* and *in vitro* PDAC models and compound screening in cancers that are in need of therapeutic options.

***Translational/Clinical Research*** ***Post-Doctoral/Medical Fellow***

# POSTER #99

## GENOMIC DESIGNS OF RAAVS CONTRIBUTE TO PATHOLOGICAL CHANGES IN THE LIVERS AND SPLEENS OF MICE

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Recombinant AAV (rAAV) gene therapy is being investigated as an effective therapy for several diseases including Hemophilia B. Reports of liver tumor development in certain mouse models due to AAV treatment and genomic integration of the rAAV vector has raised concerns about the long-term safety and efficacy of this gene therapy. To investigate whether rAAV treatment causes cancer we utilized two mouse models, inbred C57Bl/6, and Hemophilia B Balb/C mice (HemB), to test if injecting a high dose of various rAAV8 vectors containing or lacking hFIX transgene, a Poly-A sequence, or the CB or TTR promoter triggered liver fibrosis and/or cancer development over the course of the 6.5month study. We observed no liver tumors in either mouse cohort regardless of rAAV treatment through ultrasound imaging, gross anatomical assessment at sacrifice, and histology. We did, however, detect differences in collagen deposition in C57Bl/6 livers and HemB spleens of rAAV-injected mice. Pathology reports of the HemB mice revealed many pathological phenomena, including fibrosis and inflammation in livers and spleens across different AAV-injected HemB mice. Mice from both cohorts injected with the TTR-hFIX vector demonstrated minimal adverse events. While not tumorigenic, high dose of rAAVs, especially those with incomplete genomes, can influence liver and spleen health negatively that could be problematic for cementing AAVs as a broad therapeutic option in the clinic.

***Translational/Clinical Research*** ***Post-Doctoral/Medical Fellow***

# POSTER #100

## TARGETING GLUTAMINE-GLUTATHIONE-ROS AXIS TO ENHANCE CHECKPOINT IMMUNOTHERAPY EFFICACY IN COLORECTAL CANCER

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Colorectal cancer (CRC) is the second-most and third-most common cancer in women and men worldwide. CRC has become the leading cause of cancer deaths, accounting for approximately 10% of cancer-related mortality in Western countries due to an aging population, poor modern dietary habits, and increased risk factors. The development of effective treatments for CRC patients is urgently needed. Using the Inference of Cell Types and Deconvolution (ICTD), a next-generation deconvolution method for accurate assessing cell population and activities in the tumor microenvironment, we have identified the glutaminase (GLS) expression levels in the TCGA CRC database, were negatively correlated with T cell infiltration/cytotoxicity. This led us to hypothesize that targeting GLS might boost anti-tumor immune responses in CRC and potentially synergize with immune checkpoint blockade (ICB) therapy. To accomplish this, we exploited ovalbumin (OVA)/OT-I T cell-based in vitro cytotoxicity assay and CRC patient-derived organoids (PDOs)/autologous T cell killing assay and proved shRNA-mediated gene silencing or pharmacological inhibition of GLS greatly enhances $CD8^+$ T cell cytotoxicity *in vitro*. mRNA-seq screens identified antigen processing and presentation of endogenous peptide antigen via major histocompatibility complex (MHC) class I pathway was upregulated upon silencing/inhibition of GLS in tumor cells. Mechanistically, we have identified that inhibition of GLS increases levels of reactive oxygen species (ROS) by disrupting glutathione (GSH) production. Enhanced ROS activates p38-MAPK and therefore boosts JAK/STAT signaling pathway which further activates immunoproteasome genes expression. We next found inhibition of glutamate-cysteine ligase catalytic subunit (GCLC), a key enzyme in GSH production downstream of GLS, showing the same effects as GLS inhibition, which increases tumor cell MHC class I antigen presentation and synergizes anti-PD-1 treatment efficacy in mouse tumor-baring model. The discovery underscores the power of intervening metabolic pathways in combination with checkpoint blockade immunotherapy in CRC.

***Translational/Clinical Research*** ***Post-Doctoral/Medical Fellow***

# POSTER #101

## CYTOCHROME P450 OXIDOREDUCTASE (POR) ASSOCIATED WITH SEVERE PACLITAXEL-INDUCED PERIPHERAL NEUROPATHY IN WHITE PATIENTS FROM THE ADJUVANT BREAST CANCER TRIAL E5103

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Taxane-induced peripheral neuropathy (TIPN) is one of the most important factors affecting survivorship of breast cancer patients, often leading to dose reductions and premature cessation of treatment. In this study, we conducted whole-exome sequencing (WES) to detect rare variants associated with a risk of TIPN in white patients from the adjuvant, randomized phase III breast cancer trial, E5103.

WES analyses were conducted in 340 white patients from E5103 who had received a standard dose and schedule of the taxane paclitaxel. Cases (n = 168) were defined as patients who experienced grade 3 or higher TIPN. These were compared to control patients (n = 172) who were optimally matched based on demographic factors and had not developed TIPN. Only variants with a minor allele frequency ≤ 3% that were predicted to be deleterious by protein prediction programs were included in the analyses. SKAT was utilized to perform gene-based case-control analyses in order to identify genes containing a disproportionate number of deleterious variants associated with an increased risk of developing TIPN.

Cytochrome P450 oxidoreductase (*POR*) was found to be significantly associated with a risk of developing grade 3 or higher TIPN (p-value = $1.8 \times 10^{-6}$), with six variants identified in the study population that were predicted to be deleterious. Paclitaxel, a commonly used taxane, is metabolized by members of the cytochrome P450 family, and cytochrome P450 oxidoreductase is a requisite component of this process. Therefore, we hypothesize that the rare variants in *POR* alter the catalytic activities of the P450 family of enzymes responsible for metabolizing paclitaxel, leading to a higher risk of developing TIPN after exposure to taxanes. Additional analyses were conducted to determine if cytochrome P450 (CYP) metabolizer status is correlated with the development of severe TIPN. Metabolizer status of *CYP2C8*, *CPY3A4*, and *CYP3A5* was determined, and an additive logistic regression model was used to test for any association with TIPN. No significant associations were detected. Future work will be conducted to functionally validate the association of *POR* and TIPN, as well as to develop strategies that may ameliorate the impact of these variants.

***Translational/Clinical Research*** ***Research Technician***

# POSTER #102

## MULTI-OMICS ANALYTIC PIPELINE OF PEDIATRIC AND AYA SOLID TUMOR PATIENT-DERIVED XENOGRAFTS REVEALS BIOMARKERS OF THERAPEUTIC SENSITIVITY AND RESISTANCE

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Pediatric cancers, which account for approximately 1% of new cancer diagnoses each year, remain the leading cause of disease-related mortality in children. Among these cancers, solid tumors account for ~60% of pediatric, as well as adolescent, and young adult (AYA) cancers. These pediatric solid tumors encompass cancers such as: Osteosarcoma (OS), the most common, malignant bone cancer in pediatric and the AYA population; Rhabdomyosarcoma (RMS), the most common soft-tissue sarcoma in children, and Wilms' tumor, the most common renal cancer in children. Notably, prognosis remains quite poor for those patients with progressive disease underscoring the need for new therapeutic approaches that are efficacious and safe. To improve survival and quality of life in these patients, precision genomics has emerged as a valuable tool for revealing potential mechanisms of disease and therapeutic options. We developed an integrated multi-OMICs pipeline that includes analysis of the genome, transcriptome, and proteome to identify, cross-validate, and prioritize target-selection for testing in patient-derived xenograft (PDX) models. The establishment of PDX models provides an opportunity to expand tumor specimens from patients so that genomically-guided therapies can be tested. The integrated multi-OMICspipeline was used to identify therapeutic targets and understand to what extent the molecular signatures of a panel of PDX remain stable or evolve following serial passaging in immunodeficient mice. PDX models derived from naïve and pre-treated sarcoma or Wilms tumor patients were established via direct implantation of tumor specimens into NOD/SCID/γnull (NSG) mice. The original tumor and corresponding PDX passages were evaluated by the multi-OMICs approach including whole genome sequencing, RNA-seq, reverse phase protein array, and pathway enrichment analyses. Major tumorigenic gene and pathway alterations were validated, and potential therapeutic targets identified which included hyperactivation and dysregulation of oncogenic pathways including CDK4/6, MEK/ERK, PI3K/mTOR, Wnt/β-catenin, Notch, and Bromodomain and extraterminal domain (BET) proteins. While some divergence between original tumor and the respective PDX was evident at different levels of DNA analysis, cancer-associated genes and oncogenic pathways were retained in the majority of PDXs derived from sarcomas and Wilms tumors following serial passaging. CDK4/6 pathway hyperactivation and replication stress were prioritized as high-risk signatures for proof-of-concept in-vivo validation in osteosarcoma PDXs derived from patients with progressive and metastatic disease. Small molecule inhibitors to these targets significantly decreased OS tumor growth providing validation of a targeted anti-tumor response but also highlighted the need for combination therapy. Through multi-OMICsanalyses, we not only identified actionable pathways in PDXs but also validated the fidelity of the molecular signatures of PDX models following serial passaging compared to the original tumor specimens. PDX that retain the original oncogenic molecular signature will help prioritize targets for development of efficacious and safe therapies that can ultimately be translated to the clinic.

***Translational/Clinical Research*** ***Research Technician***

# POSTER #103

## DEVELOPMENT OF A NOVEL SMART AGENT THAT ELICITS A TUMOR-SELECTIVE "KISS OF DEATH" IN PANCREATIC CANCER

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Cancer is the second leading cause of death worldwide. In 2022, the American Cancer Society predicts that there will be over 1.9 million diagnosed cases and 609,360 deaths in the United States alone. Pancreatic ductal adenocarcinoma (PDAC) is an aggressive and deadly cancer that accounts for over 90% of pancreatic cancer cases and is expected to become the second leading cause of cancer related deaths. PDAC is lethal due to the lack of effective therapies and early diagnostic tools. Although there are numerous treatments today, it is necessary to develop tumor-selective therapies based on predictive biomarkers. Most chemotherapies are non-specific – killing both cancer and rapidly proliferating healthy cells. In addition, higher doses are utilized, which causes serious side effects, drug resistance, and secondary malignancies in the future. With this, one approach to reducing host toxicity on normal cells is by creating treatment agents that are activated only under tumor-specific conditions. We call these *SMART* (Selective Method of Activation for Response in Tumors) therapeutic agents. The ability to selectively target cancer cells will have a tremendous impact on precision medicine. Compared to normal cells, one exclusive feature of cancer cells is their abnormally higher content of reactive oxygen species (ROS) in the form of hydrogen peroxide ($H_2O_2$) due to little or no expression of Catalase (CAT), which is an enzyme responsible for neutralizing $H_2O_2$ levels. Thus, we hypothesize that the higher level of $H_2O_2$ in tumors can be a therapeutic advantage for the development of SMART $H_2O_2$-activatable interstrand/intrastrand DNA cross-linking agent for tumor-selective "kiss of death". Healthy cells will be protected due to higher CAT expression that efficiently removes $H_2O_2$. Here, we developed a SMART DNA crosslinking agent, eRxLinker, which is activated by $H_2O_2$. Using immunohistochemistry, we noted the presence of higher ROS in PDAC compared to normal pancreas from patients. Bioinformatics and Western blot showed significantly higher CAT expression in normal versus PDAC. Using 2D long-term DNA survival assays and 3D spheroid co-culture models, we found that eRx selectively killed cancer cells and spared normal cells. Overall, our studies should lead to the development of SMART anti-cancer agents and innovative treatment strategies to improve the overall quality of life for patients with PDAC.

***Translational/Clinical Research*** ***Research Technician***

# POSTER #104

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