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POSTER #1

RAN-REGULATED KINESIN-14 MICROTUBULE CROSS-LINKING AND SLIDING PROMOTES CENTROSOME CLUSTERING

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Centrosome amplification is a hallmark of many types of cancer that correlates with poor patient outcomes. It is estimated that in breast cancer, centrosome amplification occurs in 75-100% of solid tumors. Cancer cells with centrosome amplification form multipolar spindles but survive by clustering their centrosomes into two poles to divide their DNA into two viable daughter cells. The human Kinesin-14 HSET is proposed to provide a motorized force in centrosome clustering, as HSET depletion results in multipolar divisions leading to daughter cell death. This kinesin family cross-links and slides microtubules (MTs) through their motor and tail domains. How cells utilize Kinesin-14 mediated MT cross-linking to cluster centrosomes is unknown. The RanGTP gradient with its effectors, importin α/β, spatially regulates the cross-linking activity of Kinesin-14s. We identified two MT binding domains (MBDs) within the *Xenopus* Kinesin-14 tail that have different MT binding properties and are differentially regulated by importin α/β. MT binding of MBD1 was inhibited by the importins, whereas the MT binding of MBD2 was stronger and insensitive to the importins. To understand the roles of these MBDs in Kinesin-14 MT cross-linking and sliding in cells, we mutated the MBDs individually and together to disrupt their MT binding and then performed knockdown/rescue experiments in cells with expression of wild-type (WT), MBD1mut, MBD2mut, and MBD1-2mut lentiviruses. HSET knockdown reduces spindle length, and the WT and MBD1mut proteins rescued spindle length, whereas MBD2mut and MBD1-2mut did not. This difference in ability to rescue spindle length was not dependent on spindle localization because the WT protein localized to metaphase spindles like endogenous HSET with enrichment on MTs near the chromatin, whereas all three MBD mutant proteins had MT localization that was shifted toward the poles. To test the ability of the MBD mutants to cluster centrosomes, we knocked down HSET and treated cells with cytochalasin B to induce centrosome amplification, which increased multipolar anaphase spindles about three-fold relative to control. Transduction of the WT or the MBD2mut viruses allowed the cells to cluster their centrosomes and rescue anaphase spindle bipolarity to control levels, whereas the MBD1mut and MBD1-2mut viruses could not. These results suggest that Ran-regulated release of the importins from MBD1 is key for Kinesin-14 MT sliding needed for centrosome clustering, and MT cross-linking through MBD2 is needed for spindle length control in mitotic cells, highlighting a separation of function for Kinesin-14 activity.

Basic Science ***Assistant Research Professor***

POSTER #2

INHIBITION OF SOS1-KRAS INTERACTION BY PAN-KRAS INHIBITORS DEMONSTRATES ANTITUMOR EFFICACY IN PRECLINICAL MODELS OF CHOLANGIOCARCINOMA

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Cholangiocarcinoma (CCA) is a highly aggressive biliary tract cancer categorized into three subtypes: intrahepatic (iCCA, 10-20%), perihilar (pCCA, 50-60%), and distal (dCCA, 20-30%). CCA is associated with a particularly grim prognosis, resulting in a 5-year survival rate ranging from 5 to 15%. The standard chemotherapy regimen for CCA, gemcitabine plus cisplatin (GemCis), demonstrates dismal clinical activity. Different CCA subtypes manifest diverse genetic compositions, with commonly mutated genes encompassing *KRAS, EGFR, TP53, IDH, FGFR, BRAF, PIK3CA*, and *BAP1*. *KRAS* mutations rank among the most prevalent alterations in CCA, occurring in 7-49% of patients. *KRAS*mutation has been correlated with a more aggressive tumoral pattern and shortened survival in CCA. Various *KRAS*mutations display differing frequencies across different cancer types, with G12C mutations being rare in pancreatic cancer (2-3%) or CCA (8%), yet predominant in NSCLC (43%). FDA-approved KRAS inhibitors, sotorasib and adagrasib, specifically target the KRAS^{G12C}-mutated form, holding promise for NSCLC but not for CCA. Notably, the most common *KRAS* mutations in CCA include G12D (~48%), G12V (~18%), and G12S (~12%). Therefore, the current focus has transitioned to the exploration and development of direct $KRAS^{G12D}$-isoform specific or pan-KRAS inhibitors. We evaluated two pan-KRAS inhibitors, BI-3406 and BAY-293, which target the SOS1 protein, an activator of KRAS, in cholangiocarcinoma preclinical models. *In vitro* cell proliferation and protein expressions were analyzed using WST-1 assays and Immunoblotting, respectively. Tumor growth experiments were performed in CC-LP-1 subcutaneous xenografts. *In vitro* cell proliferation decreased with BI-3406 and BAY-293, and an additive effect was observed with the combination of these inhibitors and GemCis. The percent inhibition in cell proliferation at the low concentration range (Gem 100 nm, Cis 100 nm, BI-3406 1 μM, BAY-293 1 μM) after treatment with GemCis, BI-3406, BAY-293, GemCis + BI-3406 and GemCis + BAY-293 was: 64.57%, 35.43%, 33.06%, 72.00%, 73.80% (EGI-1 CCA cells); -6.65%, 18.95%, 19.10%, 12.34%, 49.42% (TFK-1 CCA cells); 36.8%, 13.97%, 26.05%, 49.80%, 59.39% (CC-LP-1 CCA cells); 72.68%, 36.10%, 60.06%, 87.65%, 93.70% (MMNK-1 cholangiocyte cell line), respectively. Immunoblot analysis revealed a decrease in proteins responsible for cell growth and proliferation such as PS6, SOS1, p-ERK, p-MEK and p-AKT, and an increase in apoptosis-related proteins cleaved-caspase-3, cleaved-Parp-1, and Bax. In CC-LP-1 xenografts, GemCis and BI-3406 exhibited a reduction in tumor growth, and their combination had synergistic effects. Net tumor growth in the control, GemCis, BI-3406, and GemCis + BI-3406 was 78.6 mm^3, 25.7 mm^3, 21.6 mm^3 and 6.2 mm^3. IHC analysis of tumor tissues revealed that GemCis and BI-3406 effects on tumor cell proliferation and apoptosis corresponded with tumor growth data. This investigation decisively demonstrated the antitumor efficacy of pan-KRAS inhibitors in CCA preclinical models, suggesting that combination therapies involving pan-KRAS inhibitors hold promise for improving clinical CCA therapy.

Basic Science ***Faculty***

POSTER #3

A NOVEL ISOFORM SELECTIVE HSP90BETA INHIBITOR TO COMBAT ESOPHAGEAL ADENOCARCINOMA

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Background: Inhibition of the heat shock protein 90 (Hsp90) chaperone activity with small molecules is an attractive therapeutic approach for cancer treatment because more than 300 client proteins are involved in aggressive cancer growth and metastasis. To date, ~20 inhibitors have undergone clinical trials, however, all have failed due to pan-inhibitory activities and insufficient isoform selectivity. In this study, we have investigated isoform-selective Hsp90b inhibitors, NDNB21 and NDNB11.82 on esophageal adenocarcinoma (EAC), which is one of the most aggressive human cancers with increasing incidence in the United States. A major target for EAC therapies is the human epidermal growth factor receptor 2 (Her2), which is a well-known client protein of Hsp90. In this research, we tested the effect of NDNB21 on Her2 overexpressing EAC cell lines, including one cell line that acquired lapatinib resistance.

Methods: We first evaluated the Her2 expression status by Western blot analysis in a panel of EAC cell lines (Flo-1, ESO26, OE19, OE33, SK-GT-2, ESO51, OACM5.1C, and LPR-OE19). Lapatinib-resistant OE19 (LPR-OE19) cells were established from OE19 cells by intermittent exposure to increasing concentrations of lapatinib for a period over six months. Lapatinib is a potent ATP-competitive inhibitor that simultaneously inhibits both EGFR and Her2. Antiproliferative activities exhibited by Hsp90b inhibitors (NDNB21 and NDNB11.82) on Her2 overexpressing EAC cells (OE19, OE33, and LPR-OE19) were measured by WST-1 assay. Western blot assays were performed to analyze the effect of the inhibitor on Her2 expression and to evaluate apoptosis.

Results: Both NDNB21 and NDNB11.82 dose-dependently inhibited *in-vitro* cell proliferation of Her2 overexpressing EAC cell lines with NDNB21 maintaininghigher potency. All three Her2 overexpressing EAC cell lines showed significant sensitivity to NDNB21-mediated inhibition of cell proliferation with IC_{50} values between 100 nM and 2 μM. NDNB21 dose-dependently induced apoptosis as measured by the expression of cleavage of poly ADP-ribose polymerase (cleaved PARP). Treatment with increasing doses of NDNB21 resulted in a dose-dependent decrease in Her2, p-Her2, Akt, and p-Akt. In these experiments, although the expression of pro-survival Hsp70 is induced, Hsp90 levels did not change.

Conclusions: In conclusion, our data shows that NDNB21 exhibited potent apoptotic activity against Her2-positive as well as lapatinib-resistant EAC cells, suggesting further investigation of this inhibitor in animal models and with HER2-positive EAC and ADME investigation of this new drug is warranted.

Basic Science ***Faculty***

POSTER #4

THE PNPLA3 COMMON VARIANT (I148M) EXACERBATES ALCOHOL-RELATED HEPATOCELLULAR CARCINOMA DEVELOPMENT

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Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer in adults and is one of the leading causes of cancer death worldwide. Alcohol use is a major problem globally and is a common cause of liver disease ranging from hepatic steatosis, hepatitis, cirrhosis, and HCC. Currently, how alcohol is involved in the development of liver cancer is not fully understood. It has been shown that a combination of direct hepatocyte damage by alcohol and certain genetic variations in the human genome which are involved in the hepatic metabolism are thought to play an important role. Among those genetic risk factors, the patatin-like phospholipase domain–-containing 3 (PNPLA3) variant that replaces isoleucine (I) at 148 with methionine (M), which has been identified as a major determinant of susceptibility towards a broad spectrum of liver diseases including cirrhosis and HCC after heavy alcohol consumption. Our preliminary studies have revealed that the PNPLA3148M variant plays a critical role in the development of alcohol-related liver cancer in an animal model. The humanized PNPLA3148M transgenic mice had significantly higher liver cancer incident rates and larger tumor sizes than that in control wild-type (WT) mice after 12 weeks of alcohol feeding. Further biochemical analyses uncovered a dysregulation of the Hippo pathway in the PNPLA3148M livers. Additionally, hepatic macrophage populations are altered in the PNPLA3148M mice. However, the underlying mechanisms of how PNPLA3148M is involved in these modifications remain elusive. In this application, we will further investigate the mechanisms of how the PNPLA3148M variant contributes to alcohol-related HCC development through the regulation of the Hippo pathway. We believe that the PNPLA3148M variant is a potential novel target for treating alcohol-related HCC. This proposed research is highly significant as approximately 20% of the world population carries the PNPLA3148M variant. We strongly believe that the findings from this research will provide important knowledge for the development of effective therapeutics for alcohol-related HCC patients who carry the PNPLA3148M variant.

Basic Science ***Faculty***

POSTER #5

ELUCIDATING SIMILARITIES/DIFFERENCES IN STRUCTURE AND FUNCTION OF HSP60 WITH AND WITHOUT ITS 26 AMINO ACID MITOCHONDRIAL TARGETING SEQUENCE STILL INTACT.

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Human HSP60 is transported to mitochondria by a 26 amino acid N-terminal mitochondrial targeting sequence (MTS), where, following MTS cleavage, it forms a functional toroidal oligomer. Mis-regulation of HSP60, characterized by its over-expression and cytosolic accumulation, has been identified in many cancers and is associated with promoting tumor survival, aggressiveness, and poor prognosis through modulating several oncogenic pathways. Developing drugs that exploit the aberrant HSP60 in the cytosol (ctHSP60) of cancer cells could present a targeted therapeutic approach, potentially reducing off-target effects on HSP60 in the mitochondria (mtHSP60) of healthy cells. Selectively targeting ctHSP60 may be achievable due to its distinct sub-cellular localization, differences in client proteomes, and potential structural and functional divergence from mtHSP60. Despite these prospects, therapeutic development has been hindered because the characteristics of aberrant ctHSP60, which putatively retains the MTS, remain poorly understood. We have therefore been focusing efforts on elucidating how the presence of the MTS may alter ctHSP60's structural and functional properties compared to mtHSP60.

Using analytical size exclusion chromatography, we found that ctHSP60 eluted as a mix of double and single-ring complexes, whereas mtHSP60 eluted as monomers. However, the ability of mtHSP60 to form canonical rings was supported by its HSP10-mediated folding of denatured malate dehydrogenase (dMDH) client protein. Although mtHSP60 and ctHSP60 exhibited comparable ATPase activities – with stimulation observed in the presence of HSP10 – ctHSP60 did not facilitate the refolding of dMDH in an HSP10-mediated manner. Further examining the basis of this client folding defect, we employed cryoEM to elucidate the structure of ctHSP60. In striking contrast to the double-ring structures formed by mtHSP60 and *E. coli* GroEL, where the equatorial domains constitute the ring-ring interfaces, ctHSP60 alone adopted an inverted double-ring complex where its apical domains formed the interface. However, in the presence of ATP, ctHSP60 was capable of forming 'football' complexes, similar to those reported for mtHSP60-HSP10, with HSP10 bound to either end of the properly oriented HSP60 double-ring. Thus, the underlying cause of ctHSP60's inability to facilitate client protein folding in a canonical manner remains a mystery at present. These intriguing findings prompt our continued investigation to elucidate whether ctHSP60 may play a more complex role in cancer progression than simply as a canonical chaperonin that refolds client proteins in a new environment. This line of inquiry opens avenues for cellular studies aimed at deepening our understanding of ctHSP60's function in cancer and exploring its viability as a chemotherapeutic target.

Basic Science ***Faculty***

POSTER #6

HEAD AND NECK CANCER PROMOTES BONE DETERIORATION IN AN AGE-DEPENDENT MANNER

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It is estimated that 660,000 people will be diagnosed with Head and Neck Cancer (HNC) worldwide in 2023. Notably, 40% of HNC patients will develop debilitant musculoskeletal alterations that reduce QoL and survival. Bone deterioration can occur in HNC patients in the absence of bone metastasis (1). Unfortunately, the mechanism(s) and the consequence of these bone alterations are unknown. This study is aimed to elucidate the bone deterioration in a model of non-bone metastatic HNC.

C57BL/6J male mice (2-and-13-month-old) were subcutaneously injected with 3×106 MLM3 and MLM5 HNC cells. Only the MLM3 growth caused severe loss of trabecular and cortical bone as suggested by the reduction of the Tb.BV/TV, Tb.Th, Tb.N. and Ct.BV/TV in both age. The increased bone resorption was suggested by the high levels of circulating CTX-I detected in the MLM3 bearers. The assessment of mechanical properties of the femur reveled that HNC bearers have weaker bones. The measurement of the tibia length and the proximal growth plate suggest that bone loss in young HNC mice is also due to growth failure. We then measure the circulating levels of pro-resorptive factors. High circulation IL-6 was detected in all groups, TNFα instead was found elevated in the old mice only. RANKL was unchanged whereas OPG was surprisingly elevated in all HNC bearers. As suggested by the analysis of the MLM3&5 conditioned media, HNC cells can produce OPG. Further analysis reveals an unexpected negative correlation between OPG levels and body weight, muscle mass and Tb.BV/TV.

Our study shows that non-bone metastatic HNC has severe and age-dependent detrimental effects on bone. Further studies will be performed to understand the inability of OPG to counteract bone loss in an HNC setting. Co-treatments that maintain bone health should be used in non-bone metastatic HNC patients.

Basic Science ***Faculty***

POSTER #7

THE SYNERGY OF SUMO1 DEGRADER WITH FOLFOX IN TREATMENT OF COLON CANCER PDXS

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Colorectal carcinoma (CRC) is the second leading cause of cancer death and current adjuvant FOLFOX (5-fluorouracil and oxaliplatin) chemotherapy improved overall survival of the patients. Here, we report the synergy of FOLFOX with the lead compound HB007 of the small-molecule degrader of small ubiquitin-like modifier 1 (SUM1) on colon cancer. Using human colon cancer cell lines and cancer tissue-derived organoids, we found that HB007, oxaliplatin and 5-fluorouracil slowed down the growth of colon cancer cells as individual agents and the combination treatment of HB007 with oxaliplatin or 5-fluorouracil synergistically inhibited the cancer cell growth. Next, we treated colon cancer patient derived xenografts (PDXs) and found that the combination of HB007 with oxaliplatin or 5-fluorouracil significantly improved the efforts of each of individual drugs in suppression of xenograft progression in mice. In investigation of mechanisms, we found that the synergistic effects were in part due to HB007's downregulation of the activity of the cancer drug glucose-6-phosphate dehydrogenase (G6PD) in colon cancer therapy. The study suggests that the combination overcomes G6PD-mediated chemoresistance and thereby effectively treat colon cancer.

Basic Science ***Faculty***

POSTER #8

APE1/REF-1 DRIVES ULCERATIVE COLITIS INDUCED SYSTEMIC DEFECTS IN NORMAL AND PRE-LEUKEMIC HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Abstract: Ulcerative colitis (UC) is a debilitating, immune-mediated inflammatory disorder of the gastrointestinal (GI) tract in children and adults, mostly affecting people between the ages of 15 and 35 years, while the incidence rates of UC in the USA are steadily increasing in the elderly population. UC increases the risk of developing cancers such as colorectal cancer (CRC), endometrial and bile duct carcinoma. However, whether UC influences the initiation of myeloid malignancies has not been investigated. Importantly the impact of UC on hematopoietic stem and progenitor cells in normal and pre-leukemic settings is poorly understood. Here, we have investigated the role of UC in hematopoiesis in normal mice and in mice bearing pre-leukemic *Dnmt3a* mutation. *We hypothesized that impaired immune response governed by proinflammatory cytokines in UC induces aberrant hematopoiesis and augments myeloid malignancies.* First, we developed a chronic UC-mouse model by giving dextran sodium sulfate (DSS) to C57BL/6 wild-type mice in the drinking water. We observed that mice treated with DSS developed chronic ulcerative colitis (cUC), as indicated by diarrhea and rectal bleeding, significant decrease in the body weight and colon size, and significant increase in disease activity index (DAI). Also, cUC mice presented with increased splenomegaly and increased spleen and bone-marrow (BM) cellularity in comparison to non-DSS control group. Flow cytometry analysis of colon lamina propria showed increased infiltration of $CD11b^{+}Gr1^{+}$ neutrophils, $CD11b^{+}F4/80^{+}$ macrophages and T cell leukocytes in the colon. Analysis of hematopoietic stem cells (HSCs) and progenitor cells (HSPCs) in the bone marrow (BM) revealed reduced frequency of LT-HSCs (11.75±2.4 vs 8.34±3.2; p=0.041) in cUC mice. However, the frequency of LT-HSCs (2.67±2.4 vs 9.28±3.2; p=0.0002) was increased in the spleen of cUC mice, suggesting that cUC contributes to extramedullary hematopoiesis in the spleen. We observed increased frequency of granulocyte-monocyte progenitors (GMPs) in the BM (40.19±4.8 vs 56.52±14.6; p=0.01) and spleen (5.78±2.8 vs 15.94±13.1; p=0.0007) after DSS treatment. Surprisingly, we observed that DSS treatment leads to a significant increase in the frequency of myeloid-blast like cells ($CD11b^{+}c\text{-}Kit^{+}$), suggesting that DSS-induced colitis initiates the pre-leukemic stage in mice. Transplantation of DSS treated stem cells showed impaired long-term engraftment. These defects were associated with increased secretion of proinflammatory mediators such as G-CSF, IL-6, IL-1β, IL-17, TNF-α, MCP-1, MIG in DSS-induced cUC mice. Then we recruited $Dnmt3a^{fl/fl}Mx1Cre^{+}$ mice to investigate how cUC contributes to the function of *Dnmt3a* mutant HSCs. We injected poly I:C into $Dnmt3a^{fl/fl}Mx1Cre^{+}$ mice to generate *Dnmt3a* mutant *($D3a^{-/-}$)* mice and subsequently given with DSS similar to C57BL/6 wild-type mice. $D3a^{-/-}$ mice with cUC showed an increase in the BM cellularity, absolute number of LSKs, frequency and absolute number of myeloid-biased HPC-2 cells, frequencies and absolute numbers of LKs and GMPs in the BM and spleen than C57BL/6-cUC mice. Furthermore, we observed that the presence of myeloid-blast like cells ($CD11b^{+}c\text{-}Kit^{+}$) cells (e.g., BM: 6.06±1.1 vs 13.24±1.7; p=0.0001) was further augmented in $D3a^{-/-}$ mice upon cUC development. Next, we evaluated pro-inflammatory cells and found remarkably increased $Ly6C^{hi}$ monocytes in the spleen, $CD11b^{+}Gr\text{-}1^{+}$ neutrophils in the BM, spleen and colon, and $CD3^{+}$ T cells in the colon of$D3a^{-/-}$ cUC mice compared to normal cUC-C57BL/6 mice. These data indicate that $D3a^{-/-}$ mice had accumulated more proinflammatory immune cells during cUC, which is suggestive of accelerated colitis in $D3a^{-/-}$ mice. Since gut microbiota and their by-products regulate cellular homeostasis and apurinic/apyrimidinic endonuclease 1 (APE1) as a redox activator involved in regulating cellular oxidative stress response via activating several transcription factors, we utilized APE1/Ref1 inhibitor, APX3330 to treat cUC mice. Mechanistically, we showed that the blockade of the redox-activity of APE1/Ref-1 with the drug APX3330 inhibits the elevated expression of HIF-1α in HSPCs and reverses the HSPC dynamics in both

normal and *D3a* mutant mice under inflammatory *milieu* of cUC, including the inhibition of pro-inflammatory Ly6C^{hi} monocytes. Using echinomycin, we pharmacologically blocked HIF-1α activity and found that HIF-1α mediates its inflammatory response via IL-1r1 signaling downstream from APE1/Ref-1. We show HSCs from cUC mice bearing *Dnmt3a* mutation are functionally more impaired than controls. Importantly, blockade of the redox activity of Ref-1 rescues the abnormal HSC function of both normal and pre-leukemic HSCs. Our data highlights the significance of APE1/Ref-1/HIF-1α/IL-1r1 signaling cascade in aberrant hematopoiesis that contributes to the pathophysiological conditions of chronic UC in a feed-forward loop. This study also highlights that the APE1/Ref-1/HIF-1α pathway could serve as a novel therapeutic target in ulcerative colitis in patients bearing *Dnmt3a* mutation.

Basic Science ***Faculty***

POSTER #9

THE RETINOID X RECEPTOR AGONIST MSU42011 ENHANCES E!CACY OF ANTI-PD1 THERAPY IN A HER2+ BREAST CANCER MODEL THROUGH A CD4-IL12 AXIS

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Immunotherapy is rarely elective in breast cancer and most current therapeutic options fail to target the stromal compartment. We have developed a new small molecule, MSU42011, that induces tumor regression in experimental models of Kras-driven lung cancer and HER2+ breast cancer. Thistumor regression is dependent on a functional immune system. Here, we dissected the tumor stroma, revealing a CD4 and IL12a dependency for theanti-tumor activity of MSU42011. Cancer immunotherapy responses are mostly driven by CD8 T cells. Using a murine HER2+ tumor model, anti-CD8antibodies (200ug/mouse) were injected 24 hours before treatment with MSU42011 (100MG/KG in power diet) was started. Mice were maintained ondiet for 10 days. Anti-CD8 antibodies were also administered on days 3, 7 and 10. The combination did not a!ect the inhibition of tumor growth byMSU42011, suggesting that its anti-tumor e"cacy is not dependent on CD8 T cells. In contrast, tumors treated with MSU42011 and anti-CD4antibodies grew (p=0.0016 versus MSU42011 alone) at the same rate as tumors treated with control diet (p=0.0139 versus MS42011). Furthermore,anti-CD4 reversed inhibition of tumor growth by MSU42011 in a carcinogen-induced lung tumor model. Interestingly, MSU42011 increased the levelsof IL12 by 4x (p=0.01) and IFN by 8x (p=0.03) compared to the controls. These data suggest that MSU42011 increased Th1 CD4 T cell polarization;the increase in IL12 was lost following CD4 T cell depletion. Following depletion of IL12a with antibodies (loading dose of 1 mg, followed by 0.5mgper mouse IP), tumors treated with the combination of MSU42011 and anti-IL12a antibodies grew at the same rate as the controls. T cell engagementwith MSU42011 suggests that this small molecule is a good candidate for combination with immunotherapy. HER2+ MMTV-Neu mice with establishedtumors were treated with the combination of MSU42011 and anti-PD1 antibodies. Treatment with MSU42011 at 100mg/Kg of diet for 10 days,followed by anti-PD1 antibodies (200ug) twice weekly failed to increase survival. In contrast, 2 doses of anti-PD1 antibodies (300ug), followed bytreatment with MSU42011 increased survival by 10 days compared to controls. The tumors in the MSU42011+anti-PD1 group initially grew rapidly,with increased redness and heat, suggesting an enhanced inflammatory response. When anti-PD1 antibodies were given at a lower loading dose(200ug and then 100ug twice weekly) followed by a higher dose of MSU42011 (300 mg/Kg of diet), survival markedly increased (p=0.052). Thesefindings demonstrate that activation of RXR in the stroma can be used to increase the e!ectiveness of immunotherapy.

Basic Science ***Faculty***

POSTER #10

TARGETING GLUTAMINE METABOLISM POTENTIATES ANTIGEN PRESENTATION IN COLORECTAL CANCER

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Colorectal cancer (CRC) ranks among the most prevalent cancers worldwide, causing substantial mortality. The urgent need for effective treatments has driven research into immune checkpoint blockade (ICB) therapies, which have demonstrated significant clinical benefits across various cancer types. Despite the success of ICB therapies, a significant proportion of CRC patients exhibit resistance to treatment, prompting the identification of mechanisms that hinder therapy resistance or enhance treatment response. CRC cells display remarkable adaptability, orchestrating metabolic changes that confer growth advantages, pro-tumor microenvironment, and therapeutic resistance. One such metabolic change occurs in glutamine metabolism. In this study, we found that colorectal tumors with high glutaminase (GLS) expression exhibited reduced T-cell infiltration and cytotoxicity, leading to poor clinical outcomes. To validate these findings, we applied the ovalbumin (OVA)/OT-I T cell-based cytotoxicity assay and the CRC patient-derived organoids (PDOs)/autologous T cell killing assay. Our results indicated that inhibition of GLS significantly enhanced T-cell cytotoxicity in vitro and ex vivo. Mechanistically, we elucidated that inhibition of GLS activated reactive oxygen species-related signaling pathways in tumor cells, thereby potentially increasing tumor immunogenicity by promoting antigen presentation. The combination therapy of GLS inhibitor and ICB exhibited a superior tumor growth inhibitory effect in comparison with either of the monotherapies. These findings shed new light on clinical therapeutic strategies for CRC patients, offering a promising approach to enhance the efficacy of ICB therapy.

Basic Science ***Faculty***

POSTER #11

THE HEPARAN SULFATE MIMETIC PG545 HAS ANTI-TUMOR ACTIVITY WITH SEX DIFFERENCE IN ANTI-CACHEXIA BENEFIT

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BACKGROUND: Pancreatic ductal adenocarcinoma (PDAC) is a lethal cancer with a 5-year survival of only 11%, the lowest rate relative to all other solid tumor types. Frustratingly, despite decades of research and clinical trials, there are still no effective treatment options. Cachexia is a multifactorial syndrome, occurring in up to 85% of PDAC patients and featuring progressive weight loss mainly due to muscle and fat wasting. Cachexia leads to functional impairment, reduced tolerance to anti-tumor therapy and increased mortality. PG545, a heparan sulfate (HS) mimetic, has been shown anti-tumor activity; however, it is unknown whether sex affects the anti-tumor activity and what effect PG545 has on cancer-induced muscle and fat wasting. Given that many cytokines, increased in patients with cancer cachexia, can bind HS, the aim of this study was to assess PG545 effects on PDAC cachexia with emphasis on sex difference.

METHODS: KPC PDAC cells were injected into the pancreas of 10-week C57BL/6 male and female mice. The orthotopic tumor-bearing mice received PG545 (10 mg/kg; i.p.) or PBS as a vehicle control. Body weight and body composition were monitored, and at the study endpoint, tumor and organs were collected. Skeletal muscles were cryosectioned for evaluation of cross-sectional area (CSA). Grip strength was measured. KPC cells in culture were treated with PG545 followed by immunofluorescent staining for detection of apoptosis. C2C12 myotubes, the *in vitro* model of muscle wasting, were treated with KPC cell-derived conditioned medium (CM) and/or PG545 and analyzed for the changes in size, or for mRNA extraction, followed by qPCR and RNA sequencing. Gene set enrichment analysis (GSEA) was conducted to analyze the changes in the biological functions induced by CM and reversed by PG545 treatment.

RESULTS: PG545 treatment markedly reduced tumor mass in both males and females compared to the vehicle controls; the reduction was partly due to PG545-induced cancer cell apoptosis. Compared to the respective vehicle (PBS) controls, PG545-treated male and female mice had lower body weight, lower fat and lean mass in initial doses. However, the negative effects gradually attenuated despite continuing dosing and the tumor-bearing female mice even gained fat mass subsequently. This suggests that PG545 eventually exerts anti-cachexia benefit. Grip strength of males was weakened by PG545 but became comparable to that of the untreated males at the endpoint. However, grip strength was not weakened in females at the same time points. While the muscle CSA of male tumor-bearing mice was not completely recovered from the initial PG545-induced negative effect, the CSA of the female counterpart was not significantly differently from that of the controls. Treatment of the C2C12 myotubes with CM prepared from the KPC cell culture reduced myotube size; PG545 partly prevented the reduction. This effect is in part due to PG545 inhibition of the CM-upregulated Atrogin1, the muscle-specific ubiquitin ligase gene. GSEA reveals that PG545 treatment leads to reversal of many pathway changes induced by CM.

CONCLUSION: PG545 has strong anti-PDAC tumor activity in both male and female mice, in part through induction of apoptosis. While PG545 negatively affects cachexia in the initial treatment in both sexes, it eventually has a better protective role in females.

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Basic Science ***Faculty***

POSTER #12

SUPPRESSION OF RHO-ASSOCIATED KINASE 1 (ROCK) PROMOTES HUMAN HEMATOPOIETIC STEM CELL EXPANSION BY INFLUENCING MITOCHONDRIAL FISSION

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Umbilical cord blood (CB) derived hematopoietic stem cells (HSC) have been used to treat malignant and non-malignant blood disorders for more than 30 years, but the small numbers of HSC in CB has limited their use in human transplant protocols for adults. HSCs collected and processed under hypoxia demonstrate higher engraftment and self-repopulating capacity. In our study, CITE-sequencing of hypoxia collected HSCs revealed a dramatic downregulation of Rho-associated kinase 1 (ROCK1). To assess the role of ROCK1 and to determine if ROCK1 repression in normoxia contributes to changes in HSC functions, we are genetically and pharmacologically inhibited ROCK1 in CB HSCs by using Y27632 (ROCK specific inhibitor) or shRNA. We observed enhanced *ex vivo* expansion of rigorously defined phenotypic- and functional human CB HSCs including in primary limiting dilution transplant setting as well as upon performing secondary transplantation experiments. Decreased ROS levels and DRP1 expression were detected in the mitochondria derived from ROCK1 inhibited human CB CD34+ cells. DRP1 is a GTPase that plays a critical role in regulating mitochondrial fission and excessive mitochondrial fission is associated with increased ROS production and cell apoptosis. Consistent with the lower level of ROS and DRP1 expression, higher BCL2 and lower Caspase-3 expression were observed in ROCK1 inhibited human CB CD34+ cells, suggesting that inhibition of ROCK1 promotes human CB HSC expansion by mitigation of ROS production and apoptosis via inhibition of mitochondrial recruitment of DRP1 to trigger organelle fission. Our study provides mechanistic insight into ROCK1 related networks in human CB HSCs and provides evidence that manipulation of such networks can improve *ex vivo* expansion of CB HSC/HPC.

Basic Science ***Faculty***

POSTER #13

OXYGEN TENSION - DEPENDENT SIGNALING BY PDGFRB DRIVES RESPONSE TO TARGETED THERAPIES IN BREAST CANCER

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Current approaches to preclinical cancer research often fail to consider the negative impact of maintaining cancer cells under ambient oxygen (O_2; ~21%). This is also true for hypoxia studies that typically involves cancer cells previously grown in ambient O_2 before subsequent transfer to hypoxia. However, the tumor microenvironment is characterized by significantly lower O_2 levels. We have previously demonstrated the impact of ambient O_2 on cancer stem cell populations, signaling pathways and resistance to therapy. We developed an approach that allows us to collect and process mammary tumor tissues from transgenic mouse models and breast tissue from clinically breast cancer-free women under physioxia (3% O_2) such that they are never exposed to ambient O_2. In this study, our goal was to explore signaling pathway alterations that occur due to physioxic and ambient O_2 tensions and to determine how these pathways influence response to targeted drugs. Our studies revealed increased basal phosphorylation levels of EGFR (Y1068) in cells processed and propagated in ambient air (AA), relative to physioxia. However, EGFR's downstream signaling effectors AKT and ERK showed higher phosphorylation levels under physioxia, compared to AA, suggesting that this activation is independent of EGFR signaling. These findings correlate with decreased sensitivity of the tumor cells under physioxia to targeted drugs lapatinib and alpelisib. We then sought to examine basal, and drug induced kinome changes in tumor cells in both O_2 tensions via Multiplexed Inhibitor Beads (MIBs) kinome assay. This assay revealed significant differences in the kinome of the tumor cells under physioxia compared to AA, including increased MIB binding of PDGFRβ in physioxia. We confirmed differential phosphorylation of PDGFRβ (Y751) in physioxia via Western blotting. Transient *PDGFRB* knockdown caused a simultaneous decline in the phosphorylation of AKT and ERK under physioxia, suggesting a role for PDGFRβ in the activation of these kinases under physiologic conditions. We found that Sunitinib, a multitarget RTK inhibitor with high affinity for PDGFR, effectively decreased PDGFRβ 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. Importantly, a combination of lapatinib and sunitinib rendered tumor cells under physioxia more sensitive to treatment than with either drug alone, both *in vitro* and *in vivo*. Furthermore, single cell RNA sequencing of normal breast epithelial cells revealed unique *PDGFRB* expressing epithelial cells under physioxia with EMT signatures, that was consistent with findings in the mouse mammary tumor cells. These findings suggest that ambient and physioxic O_2 tensions differentially impact cancer relevant signaling pathways and that O_2 dependent PDGFRβ signaling influences response to targeted therapies and biological processes including epithelial to mesenchymal transition.

Basic Science ***Graduate Student***

POSTER #14

TARGETING FEEDBACK CONTROL REGULATION IN INTEGRATED STRESS RESPONSE BY TRIBBLES HOMOLOG 3 (TRIB3) IN CANCER

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The Integrated Stress Response (ISR) encompasses a family of eIF2 kinases that serve to alleviate diverse stresses, including those occurring incancers.For example, nutrient limitations created duringtumor progressioninducesGCN2 phosphorylation of eIF2 that diminishes global translation, conserving energy and nutrients that are critical for reprogramming to adaptive gene expression and survival to stress. Coincident with the repression of global protein synthesis, eIF2 phosphorylation directs selective translation of ISR genes, such as that encoding ATF4 that directly induces transcription of adaptive genes thatrestorecell homeostasis. A central theme for activation of the ISR during acute stress is that the translational and transcriptional control is self-limiting, with induction of key ISR feedback regulators that ensure that translation is not prematurely resumed. In this way, the ISR is adaptive and ensures cell survival. However, prolonged activation of the ISR and expression of ATF4 and its downstream target, the transcription factor CHOP, can alter the ISR from ansurvival pathway to one that instead triggers cell death.

Given the central role of the ISR in cell stress management and its potential for adaptive or maladaptive functions, there has been extensive recent research to address the processes by which the ISR can be targeted for cancer therapies. Our studies focus on a major ATF4 target gene- Tribbles homolog 3 (TRIB3) that is suggested to be central forfeedbackcontrol in ISR.Enhanced TRIB3 in the later phase of the ISR is suggested to be critical for inactivation of ATF4and downstream targets to curtail their expression and prevent chronic induction of the transcription phase of the ISR. In order to study the regulation and function of TRIB3in the ISR, we developed a feedback culture system involving HEK293 cells treated with the drug halofuginone (HF), which mimics nutrient depletion found in a tumor microenvironment. Specifically, HF thwarts charging of tRNA(Pro), which can be alleviated by the timed addition of proline to the culture medium. We createdHEK293 cellsdeleted for TRIB3 by CRISPR and the wild-type and TRI3 KO cells were cultured in the HF feedback model to determine the changes in expression of ATF4 and its target genes. We determined that TRIB3 directs targeted degradation of ATF4, culminating in lowered levels of CHOP. Loss of TRIB3 in the feedback model leads to prolonged high levels of ATF4 and CHOP, along with reduced cell viability that is dependent on CHOP expression. We are currently addressing the precise domains of TRIB3 and the underlying mechanism that direct the targeted degradation of ATF4.This line of investigation is important to understand the disparateroles of the ISR for survival in a nutrientdepleted environment and the strategies by which these ISR processes can be altered to optimize cancer therapies.

Basic Science ***Graduate Student***

POSTER #15

DEVELOPEMENT OF A RATIONAL LIGAND DISCOVERY METHOD TO PROBE TRANSCRIPTION FACTORS

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Transcription factors are an important class of proteins whose dysregulation is often linked to Cancer phenotypes yet have largely been considered undruggable due to large elements of disorder and lack of traditional binding pockets. In this work, MixMD simulations were utilized to identify cryptic pockets in the dynamic transcriptional coactivator Med25. This model protein is known to bind PEA3 subfamily members: ETV1, ETV4, and ETV5 and dysregulation of these protein-protein interactions have been implicated in prostate cancer, breast cancer, and Ewing sarcoma. MixMD uses organic solvents as probes with unique functional groups in molecular dynamics simulations to output occupancy maps which are then overlayed to identify pockets on a protein of interest. This technique has successfully identified known binding pockets and allosteric sites on enzymes but has not yet been applied to a highly dynamic protein such as Med25. Subsequent ligand library docking results predicted a diverse array of small molecules that could bind to the pockets, and ^{1}H, ^{15}N HSQC NMR and differential scanning fluorometry (DSF) have been employed to experimentally validate ligand binding.Utilizing these two orthogonal methods, different sets of ligands were identified as hits, meriting further controls and investigation. However, 40% and 35% hit rates were observed for ^{1}H, ^{15}N HSQC NMR and DSF, respectively**,** illustrating the success of MixMD pocket identification and ligand discovery. In thefuture, these methods can be used to further facilitate rational targeting of dynamic and/or disordered transcription factors, significantly expanding the pool of druggable targets for cancer therapeutics.

Basic Science ***Graduate Student***

POSTER #16

QUANTITATIVE ANALYSIS OF NON-HISTONE LYSINE METHYLATION SITES AND LYSINE DEMETHYLASES IN BREAST CANCER CELL LINES

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Breast Cancer remains the second leading cause of cancer death in women despite the advancement of several targeted therapies. Growing evidence suggests that the dysregulation of lysine methyl mediators contributes to cancer growth and chemotherapeutic resistance. While changes in histone methylation are well documented, there is a lack of high-throughput methods to quantify changes in non-histone lysine methylation (Kme). Recent studies by our group and others have demonstrated that antibody enrichment is not required to detect lysine methylation, prompting us to investigate the use of Tandem Mass Tag labeling for global Kme quantification while also quantifying the regulators of lysine methylation in the same workflow. Tandem Mass Tag labeling (TMT labels) based mass spectrometry was used to quantify lysine methylation from four different cell lines (n=4 replicates): MCF7 (ER+ breast cancer), MDAMB231 (TNBC), HCC1806 (TNBC), and MCF10A (non- cancerous), sans antibody enrichment. We detected 365 unique Kme sites and quantified 218 of those Kme sites across all replicates; 197 unique sites were novel. Seventy-five Kme sites were identified with differential abundances across the four cell lines (ANOVA; $p < 0.05$), revealing cell line-specific patterning. Twenty-four Kme sites were upregulated or downregulated in a specific cell type. Many of the significantly altered Kme sites occur on proteins that are active targets for breast cancer treatments, such as GPX8 and ADAR1. Differentially abundant methyl mediators (lysine methyltransferases (KMTs), lysine demethylases (KDMs), and methyllysine readers) were identified. However, less than 25% of all KMTs and KDMs were quantified, suggesting that a boosting TMT channel (Trigger Channel) would be beneficial. To boost the detection of lysine demethylases, we generated a GFP-KDM Trigger Channel. Briefly, 27 GFP-KDMs were overexpressed and immunoprecipitated from HEK293T cells. The IP'd proteins were subsequently trypsin digested, TMT labeled, and added to the Breast Cancer 16-plex. All 27 KDMs were detected in the subsequent run, and twenty-six KDMs were quantified. Eight KDMs were differentially abundant across the cell lines (ANOVA; $p < 0.05$). The inclusion of the trigger channel did not have a large impact on the total number of Kme sites quantified, allowing us to consider how the impact of KDM expression could connect with specifically altered Kme sites. Pending work will focus on adding lysine methyltransferases and lysine methyl readers to the trigger channel to allow us to quantify changes in lysine methylation signaling networks.

Basic Science ***Graduate Student***

POSTER #17

POST-TRANSCRIPTIONAL REGULATION INDUCED BY HPV 16E6 AND NFX1-123 PARTNERSHIP IN LONG-TERM CULTURE OF KERATINOCYTES

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Background: Human papillomavirus 16 (HPV 16) is the most common HPV type found in cervical, anogenital, and head and neck cancers. The HPV 16 protein E6 (16E6) binds to NFX1-123, which partners with cytoplasmic poly(A) binding proteins (PABPCs) and hijacks those proteins' functions to post-transcriptionally regulate cellular differentiation, growth, and immortalization genes and pathways. This includes hTERT, the catalytic subunit of telomerase activated by 16E6, that drives cellular immortalization in HPV-associated cancers. This study seeks to understand the impact of NFX1-123 and PABPCs on the longitudinal growth of 16E6 expressing cells, and the proliferation and differentiation pathways they collaboratively affect.

Methods: We serially transduced and selected three biologically unique human foreskin keratinocytes (HFKs) with 16E6, and then either FLAG-tagged NFX1-123 (FN123), HA-tagged PABPC4, or an empty LXSN vector control. Cells were plated at 5.0x105 cells and serially counted and collected every 3 days. Population doublings were calculated by dividing the 3-day cell count by the original plating density. Total mRNA levels of transcripts involved in proliferation (i.e., hTERT) and differentiation (i.e., NOTCH1) pathways were measured by qPCR. Protein lysates from early and late passages were utilized to measure the decay rates of these target RNA transcripts involved in proliferation and differentiation pathways.

Results: 16E6 expression was confirmed by qPCR and p53 protein degradation. NFX1-123 mRNA overexpression was confirmed by qPCR and FN123 protein by western blot. PABPC4 mRNA overexpression was confirmed qPCR and HA-tagged PABPC4 protein by western blot. In 16E6/FN123 HFKs, there was increased in hTERT expression in early passages compared to 16E6/LXSN and 16E6/HA-PABPC4 HFKs. Additional gene expression modulations are being quantified.

Conclusion: We confirmed the expression of 16E6, FN123, and HA-PABPC4 in HFK lines. We found that hTERT mRNA decay rates were decreased in the 16E6/FN123 lines compared to the 16E6/LXSN cell lines. This indicates post-transcriptional stability induced by the 16E6/NFX1-123 partnership.

Basic Science ***Graduate Student***

POSTER #18

DETERMINING THE DEVELOPMENTAL CONSEQUENCES OF NF2 GENES LOSS IN NEURAL PROGENITORS IN THE CONTEXT OF SPINAL EPENDYMOMA

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NF2-related schwannomatosis (NF2) is a tumor predisposition syndrome caused by *NF2* gene mutations and characterized by the development of multiple central nervous system tumors, including spinal ependymomas (SP-EPN). Patients with SP-EPNs suffer from various neurological deficits caused by compression of the spinal cord by the tumor, and they often present at an earlier age compared to their non-NF2 counterparts. There are currently no medical therapies for SP-EPN, as surgery remains the only way to treat this tumor. Yet, surgical resection of SP-EPNs is associated with high morbidity, especially in younger patients, like those who suffer from NF2. With previous studies showing that SP-EPNs may arise from the radial glia lineage, we hypothesize that mutations in the *NF2* gene may prevent normal radial glia cell (RGC) development, leading to small populations of persistent, RGC-like cells that serve as progenitors for SP-EPN.

We performed *NF2* CRISPR knockouts in neuroepithelial (NES) cells isolated from the hindbrain region of a human embryo. We are cloning these cells to select true knockouts and will validate through western blot, RT-qPCR, and morphological analysis. In addition to our *in vitro* studies, we have performed spatial transcriptomics on a SP-EPN sample from a patient with NF2.

Results from our spatial transcriptomics revealed cellular heterogeneity within the SP-EPN sample, as well as small areas that retained progenitor cell expression. Our CRISPR system did not create complete knockouts; however, we are cloning out our polyclonal population to isolate a pure knockout. Preliminary studies with knocked-down *NF2* have shown a trend of these cells maintaining an early neural progenitor phenotype. Further, these cells form small mounds in culture when allowed to differentiate. When cells from the differentiated mounds are split into a new culture on a monolayer, they continue to proliferate and form mounds. These *NF2* knockdown clones appear to have proliferative potential following differentiation, whereas wildtype cells primarily form neurons and other mature glial cells.

Our spatial transcriptomics data suggests that there may be a developmental hierarchy within the EPC lineage, which may arise from aberrant RGCs. Although we are still working to isolate a complete *NF2* knockout, preliminary in vitro data shows that a reduction in *NF2* may cause changes within the NES cells that allow them to retain an early progenitor phenotype. Additionally, the formation of mounds in culture seems to suggest that *NF2* may play a role in cell contact interactions, which are often seen to be altered in tumors and cancers. Together, these findings are encouraging that our NES cells may be the correct model for SP-EPN and that signaling within early neural progenitors might be prime targets for SP-EPNs driven by *NF2* mutations.

Basic Science ***Graduate Student***

POSTER #19

IL-9 ENHANCES LUNG TUMOR GROWTH THROUGH INTRINSIC AND ARG1-DEPENDENT TRANSCRIPTOMIC CHANGES IN INTERSTITIAL MACROPHAGES

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A defining characteristic of cancer is its ability to evade detection by immune cells through the generation of a myeloid cell-dependent immunosuppressive tumor microenvironment. Arginine is utilized by arginase 1 (ARG1) or nitric oxide synthase in tumor-associated macrophages to respectively elicit pro-tumor or anti-tumor activity. The balance between these enzymes is regulated by extracellular cytokines including Interleukin 9 (IL-9), a pleiotropic cytokine that can be a positive or negative regulator of tumor growth. Our lab identified that IL-9 promotes tumor development in the lung by expanding interstitial macrophage populations and inducing ARG1. Lung tumor growth is attenuated in mice with myeloid cell deficiency of Arg1. Similarly, macrophage-targeting nanoparticles containing *Arg1* siRNA can therapeutically reduce tumor burden and alter macrophage populations in the lung toward an immunostimulatory phenotype. However, an understanding of the mechanism by which IL-9R/ARG1+ interstitial macrophages drive tumor progression remains incomplete. Here, using a B16F10 lung metastasis model in mixed-bone marrow chimeric mice, we demonstrate that IL-9-responsive interstitial macrophages are intrinsically altered at the transcriptomic level toward an ARG1-dependent immunosuppressive phenotype. Furthermore, our research shows that the absence of IL-9 signaling or ARG1 expression in macrophages has a notable impact on the levels of arginine and its metabolites in the lung tissue and bronchoalveolar lavage fluid of tumor-bearing mice. These alterations subsequently lead to an enhanced anti-tumoral immune response, reflected by changes in Interferon-gamma expression in both lung macrophages and T cells. Thus, our work suggests that the IL-9R/ARG1/interstitial macrophage axis promotes lung tumor development by altering intrinsic arginine metabolism and reprogramming immune cell populations toward an immunosuppressive phenotype.

Basic Science ***Graduate Student***

POSTER #20

UNDERSTANDING A POTENTIAL INTERACTION BETWEEN HSF1 AND ERRα IN BREAST CANCER

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Breast Cancer is the most common type of cancer in women with 1 in 8 women being predicted to develop breast cancer during their lifetimes. Even after decades of ground-breaking research, new cases and deaths continue to rise. Oncogenesis can often be attributed to genetic alterations, which inadvertently lead to transcriptional dysregulation, often allowing them to successfully mitigate the effects of oxidative stress, metabolic stress, and others, ultimately giving them survival benefits.

ERRα is an orphan nuclear receptor that regulates metabolic gene expression and has been linked to both ovarian and breast cancers. Inhibiting ERRα has been identified as a potential therapeutic strategy but the underlying mechanism is not fully understood. HSF1, a master transcription regulator involved in heat shock response and protein homeostasis, has been implicated in oncogenesis and promotes cell proliferation and invasion. However, any interaction or cooperation between ERRα and HSF1 has not been investigated.

Analyses of ChIP Seq data revealed a substantial number of overlapping binding peaks and shared target genes between HSF1 and ERRα. Gene ontology enrichment revealed these shared target genes are enriched for cellular pathways frequently dysregulated in cancer, such as cell proliferation, cell junction assembly and wnt signaling, among others. Using a novel ERRα transcriptional activity signature it was observed that ERRα activity was strongly correlated with HSF1 activity in breast cancer patients. Furthermore, patients with high activities of both ERRα and HSF1 were found to have poorer prognosis.

Since HSF1 and ERRα are transcription factors, we wanted to check for any cooperativity in their transcriptional activities. We performed luciferase-based reporter assays and found HSF1 to enhance ERRα transcriptional activity and vice versa. To further understand any interaction between ERRα and HSF1, we treated HEK293FT (non-cancer cell line) and HCC1937 (triple negative breast cancer cell line) with an ERRα inhibitor (XCT790) and an HSF1 inhibitor (DTHIB). Surprisingly, a reduction in HSF1 protein after treatment with XCT790 and a reduction in ERRα protein after treatment with DTHIB was observed, further suggesting some biological interaction. Moreover, co-immunoprecipitation assay results suggest a physical interaction between HSF1 and ERRα.

Taken together, our data suggests a possible protein complex with HSF1 and ERRα that stabilizes both proteins and potentially regulates pro-cancer genes in breast cancer. Future studies will be looking at the role of this complex on oncogenesis and progression of breast cancer. Additionally, HSF1 and ERRα being regulators of stress response and metabolic homeostasis in cells, it would be interesting to study the effect of this complex in conferring therapy resistance in breast cancer. These studies will lead to identification of probable therapeutic strategies exploiting this interaction, leading to improved chemotherapeutic response in patients.

Basic Science ***Graduate Student***

POSTER #21

THE ROLE OF GENERAL CONTROL NONDEREPRESSIBLE 2 KINASE IN LEUKEMOGENESIS AND THERAPEUTIC RESPONSE TO L-ASPARAGINASE

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T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematopoietic malignancy. Despite an overall improvement of outcome after initial chemotherapies, still 20% of pediatric patients fail the current regimen due to therapeutic resistance and relapse. L-asparaginase is a hydrolyzing enzyme included in the current regimen that selectively kill ALL cells by depleting circulating asparagine, a nonessential amino acid. Previous work showed that an elevated expression of asparagine synthetase (ASNS), a rating limiting enzyme for de novo biosynthesis of asparagine, is critical to drive resistance to L-asparaginase treatment. It is known that activation of the general control nonderepressible 2 (GCN2) kinase and its downstream activating transcription factor 4 (ATF4) are key steps to induce the expression of ASNS following asparagine depletion. However, our understanding of the molecular role of GCN2 in leukemogenesis and response to L-asparaginase treatment in vivo is incomplete. Since mutations in RAS family oncogene are often found in T-ALL patient with relapse, we created a model of mouse T-ALL driven by KRas(G12D) mutation expressed in T-cells specifically. Using this model, we will determine the role of GCN2 during leukemia initiation, progression, as well as therapeutic response to L-asparaginase treatment by genetic approaches. In addition, we will test whether a small molecule inhibitor of GCN2 can improve the efficacy of L-asparaginase in this model.

Basic Science ***Graduate Student***

POSTER #22

WE'RE BREAKING UP PD-1/PD-L1 INTERACTION: GENERATION OF BACTERIA-DISPLAYED CYCLIC PEPTIDE LIBRARIES AGAINST PROGRAMMED CELL DEATH LIGAND 1 (PD-L1)

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Programmed cell death ligand 1 (PD-L1) is an immune checkpoint protein expressed by cells to prevent over-activation of T-cells and maintain immune homeostasis. PD-L1 binds to its receptor, programmed cell death 1 (PD-1), which is on the surface of T-cells, impairing T-cell activity. Some cancer cells exploit this interaction by overexpressing PD-L1 on their surface, thus preventing T-cells from attacking them. Monoclonal antibodies (mAbs) that inhibit the PD-1/PD-L1 interaction have shown efficacy as cancer therapeutics; however, mAb therapeutics are costly (>$100,000 per year, per patient) and the efficiency of the treatment decreases with time due to the body developing resistance to these antibodies. Additionally, mAbs have poor tissue penetration, and are thus unable to target cancer cells deep within tumors. Cyclic peptides that inhibit the PD-1/PD-L1 interaction could perhaps serve as a more cost-effective alternative to mAbs. Due to their smaller size, cyclic peptides display greater tissue penetration than mAbs, yet maintain high target binding affinity and specificity. Herein, we use bacteria surface display to generate and screen for novel cyclic peptides that bind to PD-L1 and inhibit the PD-1/PD-L1 interaction.

Bacteria display is a directed evolution technique that enables screening of large, combinatorial peptide libraries to identify peptides that bind to a chosen target. In this technique, peptides are displayed on the surface of *E. coli* by genetically fusing the peptides to an outer membrane protein. Peptides that bind to the target of interest can then be selected using fluorescence-activated cell sorting (FACS). Traditional bacteria display technology is constrained to peptides that contain only the twenty canonical amino acids, greatly limiting the sequence space that these libraries can be used to explore. In this study, we utilized genetic code expansion to co-translationally install diverse unnatural amino acids (uAAs) into bacteria-displayed peptides. We use uAAs containing cysteine-reactive functional groups that can undergo post-translational reaction with cysteine residues to generate cyclic peptides, directly on the surface of the cell. Here we describe our efforts to identify cyclic peptides that bind to PD-L1 using bacteria display technology.

Basic Science ***Graduate Student***

POSTER #23

CHARACTERIZING THE TRANSCRIPTIONAL PATTERN OF TRUNCATED GLI1 IN GLIOBLASTOMA

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Glioblastoma (GBM) is the most common malignant central nervous system tumor in adults with a five-year survival rate of less than 7%. First identified in GBM, truncated GLI1 (tGLI1), an alternative splicing isoform of GLI1, has emerged as a cancer specific GLI1 variant as tGLI1 is highly expressed in both GBM cell lines and primary specimens but not in normal tissue. Despite an in-frame loss of 41 amino acids, the tGLI1 protein has retained all known GLI1 functional domains and appears to respond to sonic hedgehog in a similar manner as GLI1. However, 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 GBM 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 only 8.5% of GLI1 and tGLI1 binding sites are shared. These results suggest GLI1 and tGLI1 have even more unique binding pattens across the entire genome that previously thought. However, the underlying mechanism for why GLI1 and tGLI1 bind different regions of the genome remains elusive. 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 (mass spec). Results identified 45 GLI1 associated proteins and 52 tGLI1 associated proteins with only a 29% overlap between the two. Furthermore, gene ontology analysis revealed 43% of the tGLI1-specific associated proteins are involved in processes related to RNA binding, processing, and metabolism. From these RNA-related proteins, we validated an interaction between tGLI1 and the protein NONO. NONO, which can bind both RNA and DNA, is involved in a range of molecular processes including paraspeckle formation and transcriptional regulation. In GBM, high NONO expression correlates with poor patient outcomes and the mesenchymal subtype. Our results show tGLI1 interacts with and enhances NONO nuclear localization to a greater degree than GLI1. Additionally, NONO appears to increase tGLI1 transcriptional activity and exacerbate tGLI1-driven phenotypes. These effects could indicate a potential role for NONO in the differential binding pattern between GLI1 and tGLI1.

Basic Science ***Graduate Student***

POSTER #24

ONCOGENIC ETS TRANSCRIPTION FACTORS AVOID REPRESSION BY EZH2 AND FOXO1 IN PROSTATE CANCER AND EWING SARCOMA

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ETS transcription factors play roles throughout development, aiding in hematopoiesis, blood vessel formation and cell fate. The ETS family is composed of 28 members, all of which share an ETS DNA-binding domain. Chromosomal rearrangements that lead to the overexpression of specific ETS promote oncogenic transformation and tumor development. More than half of prostate tumors are driven by aberrant expression of an ETS protein. Our lab has demonstrated that oncogenic ETS form essential interactions with a ubiquitous RNA-binding protein, EWS, to promote prostate tumorigenesis. A similar mechanism exists in Ewing sarcoma (ES). ES tumors are driven by the expression EWS/ETS fusion proteins. This project focuses on the similarities and differences between oncogenic ETS in prostate cancer and EWS/ETS fusions in Ewing sarcoma.

We compared biological functions of the most common oncogenic ETS in prostate cancer, ERG, and the most common fusion in ES, EWS/FLI1. Both ERG and EWS/FLI1 promoted migration and clonogenic survival in normal prostate epithelial cell lines, RWPE1 and PNT2. These data suggest that the EWS-ERG complex and EWS/FLI1 fusion protein can function similarly in prostate cells. Knockdown of endogenous EWS/FLI1 in the ES cell line, A673, reduced anchorage-independent colony formation in soft agar. Rescue with exogenous EWS/FLI1 or EWS/ERG restored colony formation relative to control. Wildtype ERG was unable to rescue colony formation in A673. This suggests that ERG and EWS/FLI1 function differently in A673 cells. Pulldown assays from A673 lysates reveal that ERG interacts with EZH2 and FOXO1. Our lab has established that ERG acts as a transcriptional repressor through the formation of an ERG-EZH2- PRC2 complex. Others demonstrate that FOXO1 represses ERG activity by reducing its recruitment to the target sites. Both the N-terminal truncation of ERG and phosphorylation of ERG at S96 disrupted these interactions and rescued anchorage independent growth in A673 ES cells. These data suggest that EWS/ETS fusions avoid transcriptional repression in Ewing tumors due to loss of the N-terminus of the ETS protein.

Basic Science ***Graduate Student***

POSTER #25

EVALUATING THE DEVELOPMENT OF RESISTANCE TO KRAS INHIBITION IN AN INDUCIBLE KRAS MODEL OF PANCREATIC CANCER

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Pancreatic adenocarcinoma (PDAC) is presently the 3rd leading cause of cancer death in the U.S., despite being the 11th most common cancer and is further projected to overcome colorectal cancer to become the 2nd leading cause of cancer by 2030. The prognosis for PDAC is dismal, with a 5-year survival rate of 11%. The disproportionate number of cancer deaths from pancreatic cancer despite its rarity can be attributed to late-stage diagnosis and poor therapeutic options.

The genetic landscape of PDAC is characterized by pervasive oncogenic mutations in KRAS, a membrane-bound GTPase, observed over 90% of tumors. Activating mutations in KRAS lead to its constitutive activity, enhancing cellular proliferation and survival via MAPK and PI3K signaling. 98% of KRAS mutations occur at mutational hot spots G12, G13, and Q61, with G12D mutation being most common. This initial oncogenic KRAS mutation is considered the initiating event of carcinogenesis in PDAC, driving the transition from normal epithelium to early-stage pancreatic intraepithelial neoplasm.

Given the central role of KRAS in PDAC disease, targeting KRAS is of great interest. While KRAS has historically been considered undruggable due to its small size and difficulty to bind, there have been recent successes in targeting KRASG12C by an FDA-approved inhibitor Sotorasib. Inhibition of additional KRAS mutants are undergoing preclinical development and have demonstrated promise. For preclinical studies in our lab, we are using an inducible KRAS (iKRAS) model to model KRAS activation and inhibition. This model is based on iKRAS cell lines derived from transgenic tetO_LSL-KrasG12D; p53L/+; p48-Cre mice and upon doxycycline treatment, express oncogenic KrasG12D. We have successfully implanted these cells into immunocompromised and immunocompetent mice and observed doxycycline dependent tumor progression. Furthermore, we have observed KRAS independent tumor relapse after 4-6 weeks of doxycycline withdrawal. Cell lines derived from these relapse tumors retain doxycycline responsivity but can demonstrate doxycycline independent growth in vitro and in vivo compared to the parental cell line. The ability of these cells to persist despite withdrawal of KRAS provides an opportunity for further insight into the development of resistance to KRAS inhibition in pancreatic cancer.

Basic Science ***Graduate Student***

POSTER #26

INVESTIGATING THE ROLE OF KRUPPEL-LIKE FACTOR 10 (KLF10) IN PANCREATIC DUCTAL ADENOCARCINOMA (PDAC) ASSOCIATED CACHEXIA

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Cancer cachexia is a multifactorial wasting syndrome which entails muscle mass loss with or without the loss of adipose tissues. There is variability of diagnosis of cancer cachexia with specific cancer types as some types consistently have patients diagnosed than others. Pancreatic cancer, which causes the 3rd highest rate of cancer associated deaths, has the highest incident rate of cancer cachexia where up to 80% of patients are diagnosed. Previously published studies using multiple murine models of cancer show that inhibition of TGF-β superfamily members can inhibit cancer-associated muscle wasting. However, clinical trials testing anti-TGF-β therapies have not yielded positive results and adverse events/side effects remain a concern, hypothesizing that downstream targets may be better/more precise targets for potential therapeutic intervention. Kruppel-like factor 10 (KLF10) is a known downstream target of TGF-β which has been proven to affect the metabolism and development of muscle. Our work shows Pancreatic Ductal Adenocarcinoma (PDAC) tumor bearing KLF10 null mice showed a significant decrease in muscle wasting compared to their wildtype counterparts as well as suppression of atrophy specific ubiquitin ligases, Trim63 and Fbxo32. Furthermore, a ChIP-qPCR experiment demonstrated binding of KLF10 to both previously mentioned ubiquitin ligases providing evidence that KLF10 plays an important role in changes to muscle mass in tumor bearing mice. Based on previous data and current findings, KLF10 provides an intriguing target for studying cachexia and a potential target for therapeutics in PDAC patients. The scope of this project is to I) understand the regulation of KLF10 expression via the TGF-β signaling pathway, II) define the effect KLF10 has on muscle atrophy programs, and III) elucidate a target for novel therapeutics to help those suffering from cancer cachexia.

Basic Science ***Graduate Student***

POSTER #27

ERR AND HSF COOPERATIVELY REGULATE CELLULAR METABOLISM

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Metabolism must be precisely regulated to provide appropriate energy and building blocks for cellular homeostasis, proliferation, and differentiation. Inappropriate alterations in metabolic flux underlie a myriad of human diseases and play a prominent role in cancer cells. A key regulator of cell growth and metabolism is the nuclear receptor family of transcription factors, which coordinate gene expression programs with nutrient abundance and environmental cues. Our lab has shown that the *Drosophila* ortholog of the estrogen-related receptor (dERR) family of nuclear receptors is a master regulator of aerobic glycolysis – a specialized form of carbohydrate metabolism that promotes the growth of both *Drosophila* larval tissues and cancer cells. However, the ***mechanisms that govern ERR regulation in the context of aerobic glycolysis and cancer cell metabolism are poorly understood***. In preliminary work, I found mutations in the *Drosophila* ortholog of the heat-shock response factor (dHSF) family induce larval lethality and defects in glycolytic gene expression that are similar to those observed in *dERR* mutants. This result raised the possibility that dHSF is a coregulator of dERR in controlling larval metabolism, and by extension, human metabolism. Consistent with this idea, we observed a protein interaction between human HSF1 and ERRα in an ovarian cancer cell line as well as a normal, healthy human cell line. By analyzing the transcriptome of 302 ovarian cancer patients from the TCGA database, we found that both gene transcription and gene activity of ERRα and HSF1 are significantly correlated ,suggesting that there may be a conserved interaction between HSF and ERR that spans from invertebrates to mammals. Moving forward, our goal is to determine the molecular mechanisms of ERR/HSF interaction and its function in fly development and ovarian cancer progression. By leveraging the special insights offered by *Drosophila*, our long-term goal is to bridge the knowledge gap in metabolic regulatory mechanisms of ERR and HSF that are conserved across metazoan lineages and reveal new metabolic vulnerabilities within cancer cells, thereby paving the way for pioneering therapeutic avenues in precision oncology.

Basic Science ***Graduate Student***

POSTER #28

ROLE OF NOTCH3 ACTIVATION IN THE GENERATION OF OVARIAN CANCER METASTASIS PROMOTING CAF SUBPOPULATIONS

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Ovarian cancer (OC) has the highest mortality rate amongst gynecologic malignancies due to extensive metastasis. Among all OC subtypes, high grade serous ovarian cancer (HGSOC) is the most aggressiveness and predominant. The intrinsic complexity of metastasis remains elusive and difficult to target, despite causing ~90% of cancer-related deaths. The stromal components of the tumor microenvironment (TME) play critical roles in driving metastasis. Cancer associated fibroblasts (CAFs), which constitute 10-50% of the stroma, are capable of ECM secretion/remodeling, inducing tumor angiogenesis, rewiring metabolic and immune landscape, among others, making them a major contributor to the development of metastasis. However, critical questions pertaining CAF activation and generation of a pro-metastatic phenotype remain unanswered. CAFs consist of multiple transcriptionally and functionally distinct heterogeneous subtypes. The most well-recognized subtypes include the highly contractile, ECM remodeling myofibroblastic CAFs (myCAFs) and immunomodulating inflammatory CAFs (iCAFs). In HGSOC, the mechanisms that generate these CAF subtypes and their mechanisms of action remains unidentified. Using a combination of bioinformatic and *in vitro* approaches, we have studied the juxtacrine cross-talk between metastasizing OC cells and CAFs in the context of CAF activation and heterogeneity. To identify mechanisms of CAF activation by metastasizing OC cells, we analyzed the molecular subtypes of HGSOC from the TCGA dataset, which implicated upregulated Notch signaling in CAFs. Furthermore, analysis of scRNA-seq data from 11 HGSOC patient metastasis and from a heterotypic 3D coculture of ascites-derived primary HGSOC cells and CAFs was done. We identified 9 unique CAF subpopulations having differential patient distributions, reciprocity between myCAFs and iCAFs, and expressing a continuum of activated Notch reactome. By performing Notch ligand-receptor analyses, we determined Jagged1 (Jag1)-expressing HGSOC cells as initiator of crosstalks for the induction of either Notch2 or Notch3 in CAFs, in the respective datasets. Our genetic knockdown studies manipulating these axes in CAFs, indicated Notch3 as the pivotal receptor and a reciprocal relationship between Notch2 and Notch3 for the regulation of Hes1 and α smooth muscle actin (αSMA) expressingmyCAFs. Moreover, activation of Notch3 either via Jag1-mediated induction or by stable overexpression of notch intracellular domain 3 (NICD3) induced Hes1 and αSMA, indicating myCAF activation. Notch3 was crucial for the ECM remodeling functionality of activated myCAFs and for the suppression of IL-6^{+} iCAFs. High Notch3 expression in CAFs resulted in poor prognosis in HGSOC patients. In the long term, targeting this axis can potentially translate into effective therapeutics for metastatic HGSOC.

Basic Science ***Graduate Student***

POSTER #29

ELUCIDATING THE MECHANISMS BY WHICH ETV1 DRIVES PROSTATE ONCOGENESIS

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Prostate cancer (PCa) remains the most diagnosed, and second deadliest, cancer among American men, accounting for nearly 300,000 cases and 25,000 deaths in 2023. In most cases (59%) an ETS transcription factor is translocated downstream of an androgen-responsive promoter. Of these, the most common fusion is that of ERG to the *TMPRSS2* promoter (*TMPRSS2-ERG*) which is observed in 46% of all cases of PCa. In the remaining instances of ETS-positive PCa, a member of the PEA3 subfamily, namely ETV1 (9%) or ETV4 (5%) is fused. Given the infrequency of PEA3 rearrangements in PCa patients, little is known regarding the mechanisms by which these proteins drive oncogenesis.

Previous studies in the lab have extensively characterized the role of ERG using the normal prostate cell line RWPE-1. ERG expression in RWPE-1 drives cellular migration and, in the presence of AKT activation (mAKT), clonogenic growth. Likewise, injection of RWPE-ERG with mAKT, but not ERG alone, into nude mice results in tumorigenesis. In addition to phenotypic changes, the lab has also shown that ERG can drive changes in the TLR4 and VEGFA pathways via positive feedback.

Based on these findings, I have investigated the role of the PEA3 factor ETV1 in the normal prostate cell line PNT2. Given that ETV1 is normally targeted for degradation in prostate cells by the E3 ubiquitin ligase COP1, I first generated a 131 amino acid truncation of ETV1 (tETV1). This removes the COP1 binding site which is commonly lost due to translocation in ETV1-positive PCa patients. To mimic the level of ETV1 expression in PCa patients, I fused the truncated protein downstream of the constitutively active *HNRNPA2B1* promoter (*HNRNPA2B1-tETV1*).

Through these studies, I found that overexpression of tETV1 in PNT2 drives the gene expression of both TLR4 and VEGFA. However, it remains unknown whether ETV1 directly facilitates transcription of these genes, or if these pathways are upregulated by indirect mechanisms. Additionally, tETV1 drove a modest, but significant, change in the basal epithelial marker TP63, and a robust increase in phosphorylated AKT (pAKT). Upon co-expression of tETV1 and mAKT, cells (PNT2-tETV1-mAKT) retain increased TLR4 expression, but no longer demonstrate increased VEGFA expression. Unlike tETV1 alone, tETV1 with mAKT demonstrates no significant change in TP63, but still drives endogenous AKT phosphorylation.

Together, these data indicate that, when compared to ERG, ETV1 drives similar, but not identical, changes in prostate gene expression. Further elucidation of the regulation of these signaling pathways is necessary to identify therapeutic targets in patients with ETV1-positive prostate cancer.

Basic Science ***Graduate Student***

POSTER #30

IMPROVING LYMPHOCYTE RECOVERY THROUGH EX VIVO EXPANSION FOR HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Hematopoietic stem cell transplantation (**HCT**) is a potential curative therapy for both malignant and non-malignant hematologic disorders, yet many patients continue to relapse and succumb to their disease. Expeditious recovery of immune cells post HCT is a critical determinant of patient outcome, where absolute lymphocyte count by day 30 post transplantation (**ALC30**) is associated with increased rates of overall and progression-free survival and reduced rates of graft vs. host disease. However, there is currently no clinical therapy to enhance lymphocyte recovery. Improving HCT remains a critical unmet need in order to increase survival rates for patients with hematologic disease. One FDA approved clinical strategy to increase numbers of hematopoietic stem and progenitor cells for HCT is through *ex vivo* expansion. *Ex vivo* expansion of umbilical cord blood (**CB**) units has been shown to decrease recovery time of critical hematopoietic cell populations such as neutrophils and platelets, but the use of expansion to improve recovery of lymphocytes remains uncertain. Using bone marrow (**BM**) HCT as a model for CB HCT (due to limitations of humanized mouse models), we transplanted expanded lineage negative BM cells from C57BL/6 (**C57**) mice into lethally irradiated C57BL/6xBoyJ (**F1**) hosts and compared immune cell recovery in multiple organs to those of mice transplanted with unmanipulated lineage negative BM. We hypothesized that *ex vivo* expansion will increase absolute numbers of leukocytes and hematopoietic stem and progenitor cells, thereby enhancing engraftment and lymphocyte recovery and resulting in increased efficacy of transplantation. Via complete blood count, mice transplanted with expanded BM had increased lymphocyte counts compared to mice transplanted with unmanipulated BM at weeks 2 and 5 post transplantation. At week 5 post-transplant, the peripheral blood, BM, thymus, and spleen of mice were predominately composed (<70% chimerism) of C57 expanded cells. We observed increased B-cell reconstitution in the spleen, BM, and peripheral blood at weeks 2, 5, and 8 post HCT in mice receiving expanded BM compared to unmanipulated BM. T-cell progenitor subsets were increased in mice receiving expanded BM, however mature CD4+ and CD8+ T-cell percentages did not change. Host recovery, marked by F1 cells, was decreased in mice receiving expanded BM compared to unmanipulated control BM. Our data suggests that expanded BM enhances B-lymphocyte recovery at early timepoints post HCT and suppresses host recovery more efficiently than unmanipulated control BM. We did not observe increased mature T-cell recovery with use of expanded cells, which may be partially explained by the notion that thymopoiesis was only partially complete by the end of this study. Extended timepoints are needed to fully assess T-cell recovery. Taken together, expanded cells are a promising donor source for HCT and can result in augmented immune cell reconstitution. Further testing is needed to address if expanded cells can decrease risk of relapse and susceptibility to infection.

Basic Science ***Graduate Student***

POSTER #31

EXPANDING THE LIGANDABLE PROTEOME: COVALENTLY TARGETING CONSERVED RNA BINDING PROTEINS

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RNA binding proteins are essential for a variety of cellular functions including metabolism, transcription, and translation. RBPs are an important therapeutic target as they have the most known disease annotated mutations of any protein family. Targeting RBPs is particularly promising for the treatment of cancer because RBPs have been shown to drive oncogenic splicing in a variety of cancers. However, RBPs have historically been considered “unligandable” by traditional methods due to their highly dynamic and disordered nature. An additional challenge in targeting RBPs is the high level of conservation found within RNA binding domains. We hypothesized that covalent small molecules could be used to successfully and selectively engage highly conserved RBPs. To investigate this, we screened a library of cysteine-reactive covalent ligands against two structurally and sequentially conserved RBPs; heterogeneous ribonucleoproteins H and F, hereafter referred to as hnRNP H and hnRNP F. We chose hnRNPs H and F as a model system for RBP conservation because they have over 89% sequence similarity, three parallel quasi-RNA recognition motifs (qRRMS), and three conserved cysteines (C22, C34, & C122). Despite this conservation, we observed distinct differences in the covalent labeling of hnRNP H and hnRNP F during in vitro screenings. Notably, we found one small molecule with a ten-fold difference in selectivity for hnRNP H over hnRNP F. By screening covalent small molecules with various cysteine-reactive covalent warheads and varied molecular recognition moieties we began to explore how covalent bond formation provides opportunities to tune selectively and enables differentiation among highly similar proteins. This work demonstrates that covalent small molecules can successfully and differentially engage highly dynamic and conserved RNA binding proteins hnRNP H and F. Furthermore, this work has the potential to reshape our understanding of selectivity in the context of covalent bond formation and expand what we consider to be the ligandable proteome.

Basic Science ***Graduate Student***

POSTER #32

UNSCHEDULED ENDOCYCLES DISRUPT OVARIAN STRUCTURE AND FUNCTION AND IMPAIR FEMALE FERTILITY.

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Cancer therapy resistance and relapse remain poorly understood. A plethora of recent evidence indicates that polyploid giant cancer cells (PGCCs) are a major contributor to resistance and relapse. Therefore, developing a deep understanding of PGCCs would bring on a paradigm shift in cancer treatments and prevention. PGCCs arise through repeated growth and synthesis phases via unscheduled endocycling that are induced by various stressors, including genotoxic and anti-mitotic cancer therapies. Evidence from cells in culture and patient tumors suggests that, upon switching to endoreplication, PGCCs become resistant to cancer therapies and are a persistent tumor cell population. *Despite their importance, the impact of PGCCs on tissue growth and function* in vivo *is underexplored.* To address this knowledge gap, I am using *Drosophila* ovarian somatic follicle cells as a model to examine the impact of unscheduled endocycles on tissue growth and function.

Follicle cells normally switch to endocycles at mid-oogenesis. I have found that prematurely inducing this switch to endocycling can have drastic consequences for ovarian function and female fertility. Starting endocycles too early disrupted the later cell cycle arrest and transition to developmental amplification of eggshell genes in later oogenesis. These females laid eggs with thin shells and resulted in significant embryonic lethality. Further examination of these ovaries revealed an array of pleiotropic defects, including reduced oocyte growth and aberrant follicular epithelial structure. Disruption of epithelial structure is known to contribute to tumorigenesis, so this presents one avenue whereby unscheduled endocycling cells can affect tissue overgrowth. Additionally, premature endocycling inhibited the collective cell migration of a special set of follicle cells known as border cells in mid-oogenesis. As border cells are a powerful model for the collective cell migration observed in human metastases, my last result challenges current models that polyploidy enhances migration and metastasis of human cancer cells. The findings described above were recently published in the journal Genetics. I am currently working to extend these findings by using a tumorigenesis model in flies to examine how unscheduled endocycling cells can synergize with oncogenic mutations to give rise to more aggressive tumors. Overall, these findings have broader impact by revealing how unscheduled endocycles can disrupt tissue growth and function to cause aberrant development and cancer.

Basic Science ***Graduate Student***

POSTER #33

TARGETED LIPOSOMAL DELIVERY OF PI3K INHIBITING PRODRUGS TO TREAT BREAST CANCER

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Breast cancer is the most common type of cancer for women in the United States, with an estimated 287,850 new cases in 2022. Despite advances in medicine, approximately 15% of all cancer-related deaths were due to breast cancer, warranting the need for new treatments. Subpopulations of aggressive cancer cells have been shown to express a stem-like cancer marker (SLCM) on the cell surface, making this receptor a good candidate for the targeted delivery of chemotherapies. Overactivation of phosphoinositide 3-kinase (PI3K) and the subsequent PI3K/AKT/mTOR pathway has been observed in many forms of cancer and resulted in the synthesis of various PI3K inhibitors. Nevertheless, poor drug tolerance and limited maximum tolerated dosages has hampered the availability of PI3K inhibitors to patients. Our research has focused on engineering SLCM-targeted liposomal nanoparticles loaded with PI3K-inhibitor prodrug for selectively delivery to breast cancer cells. An SLCM-targeting peptide sequence was identified and synthesized for liposomal incorporation. *In vitro* studies were performed to ensure that cellular uptake of nanoparticles was enhanced by the presence of the SLCM-targeting peptide. A 30-fold enhancement in uptake was observed for BT-474 cells (HR+/HER2+) and a 13-fold enhancement was observed for SK-BR3 cells (HR-/HER2+). Cytotoxicity assays have shown an IC_{50} value of approximately 1uM for the free PI3K inhibitor, however, free PI3K inhibitor prodrug, non-targeted prodrug-loaded nanoparticles, and SLCM-targeted prodrug-loaded particles display IC_{50} values between 10-25uM. These findings have led our group to pursue different PI3K inhibitor prodrug chemistries to encourage the release of the active drug. Additionally, we are interested in investigating the cytotoxicity of SLCM-targeted nanoparticles dually loaded with the PI3K inhibitor prodrug and a maytansinoid prodrug.

Basic Science ***Graduate Student***

POSTER #34

AN UNSCHEDULED SWITCH TO ENDOCYCLES INDUCES A REVERSIBLE SENESCENT ARREST THAT IMPAIRS GROWTH OF THE DROSOPHILA WING DISC

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The regulation of polyploid cells and their impacts on tissue growth are not well understood. Polyploidy is one of the hallmarks of tumors. Roughly thirty percent of tumors in humans undergo whole-genome duplication to become polyploid. This process can be regulated through a specified cell cycle called endocycle, which allows cells to undergo repeated G/S phases without cell division. The endocycle is highly conserved as part of normal development in many different species, including humans. However, cells can also undergo an unscheduled switch to endocycles in response to aging, stress, and environmental inputs. We call these induced endoreplicating cells (iECs) to distinguish them from scheduled developmental endoreplicating cells (devECs). While iECs aid in wound healing and tumor suppression, they also contribute to cancer therapy resistance and tumor regrowth, for example, Polyploid Giant Cancer Cells (PGCCs). Upon cessation of therapy, the surviving PGCCs can resume error-prone divisions that generate more aggressive aneuploid cancer cells. Much remains unknown, however, about the regulation and growth of these unscheduled iECs and how they impact normal or pathological growth.

Using the *D. melanogaster* wing disc as a model, we found that populations of iECs initially increase in size but then subsequently undergo a heterogeneous arrest that causes severe tissue undergrowth. iECs acquire DNA damage and activate a Jun N-terminal kinase (JNK) pathway, but, unlike other stressed cells, they are apoptosis-resistant and are not eliminated from the epithelium. Instead, iECs enter a JNK-dependent and reversible senescent-like arrest. Senescent iECs promote the division of diploid neighbors, but this compensatory proliferation does not rescue tissue growth. Our study has uncovered unique attributes of iECs and their effects on tissue growth that have important implications for understanding the mechanisms of PGCC growth and survival.

Basic Science ***Graduate Student***

POSTER #35

THE RXR AGONIST MSU-42011 AND THE MEK INHIBITOR SELUMETINIB REDUCE PERK LEVELS IN NF1-DEFICIENT CELLS AND INHIBIT CYTOKINE PRODUCTION IN MACROPHAGES

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Neurofibromatosis type 1 (NF1) is a common genetic disease that predisposes approximately 50% of affected individuals to develop plexiform neurofibromas (PNFs), which can progress to highly aggressive malignant peripheral nerve sheath tumors (MPNSTs) in approximately 10% of patients. NF1 is caused by mutations in the tumor suppressor gene *NF1*, which encodes for neurofibromin, a negative regulator of RAS activity. Selumetinib, a specific inhibitor of MEK1/2, is the only FDA-approved drug for NF1-associated PNFs. However, the anti-tumor effects of selumetinib are limited in MPNSTs and have dose-limiting side effects. Deficiency of the *NF1* gene not only promotes tumorigenesis but also has broad effects on the immune cells and cytokine signaling driven by hyperactive RAS signaling. Because macrophages account for almost half of cells in NF1 lesions and their infiltration correlates with disease progression, we hypothesized that targeting tumor-promoting immune cells could be an alternative approach for treating NF1. The novel retinoid X receptor (RXR) agonist MSU-42011 reduces tumor growth in experimental Kras-driven cancers by decreasing pERK expression, reducing tumor-promoting immune cells like CD206+ macrophages and regulatory T cells, and increasing activated cytotoxic T cells. Here, we treated NF1-deficient cells and macrophages with MSU-42011 and selumetinib, either alone or in combination, using monoculture and conditioned media (CM) conditions. In human PNF cells and mouse MPNSTs, treatment with 200 nM MSU-40211 or 50 nM selumetinib for 3 hours reduced pERK protein levels compared with untreated controls, and the combination treatment enhanced this reduction in pERK protein levels. Additionally, there was a trend toward reduction in cell viability with increasing drug concentrations after 72 hours of the combination treatment. Moreover, CM from human and mouse PNF cells increased the mRNA expression of monocyte chemoattractant *CCL2* (C-C motif chemokine ligand 2) and the secretion of IL-6 and TNFα in human THP1 monocytes/macrophages and bone marrow derived macrophages (BMDM). Notably, MSU-42011 and selumetinib alone inhibited *CCL2* mRNA expression in THP1 macrophages and BMDM stimulated with CM from human and mouse PNF cells, respectively, and the inhibition of *CCL2* mRNA expression was greatest with combination treatment. The combination of MSU42011 and selumetinib also significantly reduced tumor burden in a LL2 model of lung cancer driven by an activating *Kras* mutation. Based on the similarities in RAS activation and immune cell infiltration in NF1 and lung cancer, our next step is to confirm the immunomodulatory and anti-tumor effects of MSU-42011 and selumetinib in a syngeneic model of PNF and MPNST.

Basic Science ***Graduate Student***

POSTER #37

PH-RESPONSIVE THERAPEUTIC EXOSOMES FOR ENHANCED DELIVERY OF IMMUNOTHERAPEUTIC SIRNA

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Exosomes have prominent potential as highly efficient nanocarriers for the delivery of genes for anticancer therapy. While exosomes have demonstrated a preference for uptake via receptor-ligand interaction-mediated endocytosis. Yet, a large portion of endocytosed exosomes end up entrapped within lysosomes, which facilitates their degradation. Therefore, it is essential to functionalize and engineer exosomes to efficiently escape lysosomes to achieve successful intracellular drug delivery and desired therapeutic efficacy.

In this work, we designed and synthesized GALA peptides, pH-sensitive fusogenic peptides, that can be inserted onto exosome membranes and thus enhance cytosolic delivery of exosomal cargo through lysosomal escape. Herein, we harvested exosomes from MCF-7 cell cultures due to the homing effect of exosomes toward breast cancer for targeted drug delivery. After isolation and purification of exosomes from culture media, we functionalized their membranes with as-synthesized GALA peptides. GALA-functionalized exosomes (GALA exo) exhibited a surface charge change from negative (-8.94 mV) at pH 7 (extracellular environment) to positive (+0.85 mV) at acidic pH 4 (lysosomal environment).Hence, upon cellular uptake of GALA exo via endocytosis, the pH decrease in the lysosome converted the exosomal membrane to a positive charge, facilitating fusion with the negatively charged lysosomal membrane. We have demonstrated this process by utilizing fluorescent chiral graphene quantum dots (GQDs) as representative exosomal cargo in exosomes, taking advantage of the superior permeability of chiral GQDs into lipid membranes, as well as their excellent optical properties for tracking analysis. Consequently, the results showed that the cytosolic release of GQDs, representing exosomal cargo, was 1.7 times higher than that of pristine exosomes within MCF-7 cells.

Meanwhile, chiral GQDs can effectively carry genomic drugs via pi stacking, and load the drugs into exosomes with high efficiency (>60%). Combining the advantages of intracellular cargo release via GALA peptides and the high loading efficiency of small interfering RNAs (siRNAs) via chiral GQDs, this study provides a novel gene therapy strategy to treat breast cancer.

Basic Science ***Graduate Student***

POSTER #38

THE ROLE OF PARASPECKLES IN EWING SARCOMA

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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 Ewing sarcoma, an adolescent bone cancer.In Ewing sarcoma, the intrinsically disorder region (IDR) of EWS is fused to ETS members, primarily FLI1, and promotes aberrant expression and activation. The IDR of EWS promotes self-binding, forming phase separated granules, and acts as a transcriptional activation domain. Mutation of the IDR of proteins similar to EWS have been shown to inhibit phase separation, activation, and tumorigenesis. This suggests that phase separation is linked with activation and important for tumorigenesis, however the mechanism of this is still not known. One potential mechanism is the involvement of a phase separated body. Paraspeckles are nuclear phase separated bodies that have a role in transcriptional regulation, but their role in cancer is not well understood. I have shown that EWS/FLI1 interacts with the long noncoding RNA (lncRNA) NEAT1, which is the necessary RNA scaffolding of the paraspeckle. A673 cells, an Ewing sarcoma line, show paraspeckle granule presence using NEAT1 RNA-FISH. Knockeddown (KD) of two necessary paraspeckle components, NEAT1 or FUS, in A673 cells reduce paraspeckle presence, colony growth in soft agar, and EWS/FLI1 activity at the transcriptional level. KD of FUS and NEAT1 also increased migration, which is consistent with EWS/FLI1 loss. Taken together, these data suggest that the paraspeckle has a role in EWS/FLI1 transcription in Ewing Sarcoma.

Basic Science ***Graduate Student***

POSTER #39

LSD1 AND COREST2 DEMETHYLATE STAT3 TO PROMOTE ENTEROENDOCRINE CELL DIFFERENTIATION IN MUCINOUS COLORECTAL CANCER

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Across different cancer types, tumor heterogeneity has been shown to drive tumor progression, metastasis, and therapeutic resistance. Adenocarcinoma to neuroendocrine lineage transition is an emergent mechanism of targeted therapy resistance in several cancer types, including lung and prostate cancer. Therefore, understanding the dynamics and mechanisms driving neuroendocrine cell fates in cancer is critical. Mucinous colorectal cancer accounts for upwards of 20% of colorectal cancer cases and is characterized by tumors with mucous accounting for at least 50% of the tumor volume. The normal colon epithelium consists of several specialized cell types, including hormone secreting enteroendocrine cells (EECs), which are the neuroendocrine cell of the intestine. We have previously shown that EEC progenitors are enriched in mucinous colorectal cancer and promote cancer cell survival via secreted factors. Additionally, we have shown that lysine specific demethylase 1 (LSD1) promotes EEC differentiation in these tumors; however, the mechanism by which LSD1 promotes EEC differentiation has remained unknown. Typically to carry out its enzymatic function, LSD1 must be a part of a transcriptional regulatory complex. One such complex is the CoREST complex, which contains LSD1, HDAC1, and one of three CoREST protein family members. Here we report that LSD1 and CoREST2 promote EEC differentiation by demethylating the transcription factor signal transducer and activator of transcription 3 (STAT3) to promote STAT3 chromatin binding. Additionally, we demonstrate that knocking down CoREST2 decreases tumor growth and lung metastases of mucinous colorectal cancer cells injected orthotopically into the colons of immunocompromised mice. Furthermore, we utilized single cell multi-omics that combines single cell RNA sequencing with single cell ATAC sequencing to show that during EEC differentiation there is an increase in chromatin accessibility at regulatory regions of known EEC-promoting transcription factors. Finally, through our single cell multi-omics analysis we identify a rare new cell type with neuron-like but not secretory characteristics. Mucinous colorectal cancer is an aggressive and chemotherapeutically intractable form of CRC that is enriched for EEC progenitors.Our data demonstrates that LSD1 and STAT3 promote EEC differentiation, suggesting that inhibiting LSD1 and or STAT3 may have benefits for patients with mucinous colorectal cancer.

Basic Science ***Graduate Student***

POSTER #40

UNDERSTANDING THE INTERACTIONS BETWEEN COLON CANCER EPITHELIAL AND MAST CELLS IN THE PROMOTION OF BRAF MUTANT COLORECTAL CANCER

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Colorectal cancer (CRC) represents the second most common cause of cancer-related deaths in men and women combined in the US. CRC subtypes with activating mutation in the *BRAF* gene have many molecular and phenotypic differences such as poor response to standard treatments and lower overall survival. Our long-term goal is to find better therapeutic strategies to treat mutant *BRAF* CRC. Our objective is to understand what contributes to the aggressiveness of *BRAF* mutant CRC. Our laboratory demonstrated that secretory cells are enriched in *BRAF* mutant CRC and that these cells contribute to tumorigenesis by incompletely understood mechanisms. Additionally, we have shown that mast cells (MC), a type of granulocytic immune cell, are enriched in *BRAF* mutant CRC. MCs are recruited to tumor sites and activated by tumor cell-secreted factors. Activated MCs can be either pro- or anti-tumorigenic depending on the context. Interestingly, it is known that secretory cells can interact with immune cells, including MCs under normal physiological conditions. However, whether secretory cells and MCs interact in *BRAF* mutant CRC is unknown. My preliminary results demonstrate that secretory cells enriched in *BRAF* mutant CRC are involved in the recruitment of MCs in vitro. I am, now, working to uncover the secreted factor(s) responsible for the recruitment of MCs. My preliminary results also demonstrate that epithelial-to-mesenchymal transition (EMT) is induced in *BRAF* mutant CRC cells upon direct interaction with MCs. EMT markers, such as Slug, Snail, and vimentin increase at the gene and/or protein level in CRC cells when they are cocultured with MCs. The migratory ability of *BRAF* mutant CRC cells also increases after incubation with MCs. Therefore, based on published and preliminary data, I *hypothesize* that in *BRAF* mutant CRC, secretory cells alter the infiltration and activation of MCs, which in turn promote EMT and tumor progression. Considering that secretory cells secrete various factors; I hypothesize that secretory cells are necessary for the pro-tumorigenic activation of MCs in *BRAF* mutant CRC. My work currently focuses on understanding how secretory cells recruit and activate MCs in *BRAF* mutant CRC. Additionally, I am unraveling how MCs and *BRAF* mutant CRC cells interact and how this interaction drives EMT in *BRAF* mutant CRC cells.

Basic Science ***Graduate Student***

POSTER #41

INVESTIGATION ON THE ROLE OF TUMOR SUPPRESSIVE MICRORNAS IN ANGIOSARCOMA

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Angiosarcoma is an aggressive tumor resulting in a very poor prognosis for patients. MicroRNAs (miRNAs) can regulate gene expression and play important roles in a variety of cancer. Our previous studies demonstrate miRNA loss leads to angiosarcoma in mice. To further understand the role of miRNAs that function as critical tumor suppressors in angiosarcoma, in Aim 1, we performed a miRNA-focused CRIPSR-Cas9 screen. The gRNA library was transduced into human angiosarcoma cell lines expressing doxycycline (dox)-inducible Cas9. SgRNA amplicon sequencing on DNA samples was performed to determine the change in the frequency of gRNAs. Three miRNAs were identified as hits with significant enrichment of multiple gRNAs, including miR-200b, miR-181b, and miR-410. I have begun the functional validation studies by overexpressing these miRNAs in a panel of angiosarcoma cell lines. In preliminary results, the overexpression of miR-410 showed consistent effects on inhibiting cell proliferation and colony formation ability in multiple angiosarcoma cell lines. Additionally, to understand miRNAs-related mechanism, in Aim 2, a novel technique called AgoTRIBE was used to identify important mRNA targets regulated by miRNAs. In AgoTRIBE, miRNA effector Ago is fused with a RNA editing protein ADAR and leads the fusion protein to the natural targets of miRNAs. The ADAR editing events on miRNA targets can be detected by RNA seq. We used a dox-inducible lentiviral vector expressing human ADAR (hADAR) or hADAR-Ago in a mouse angiosarcoma cell line. In preliminary data, we validated the protein expression of ADAR and ADAR-Ago upon dox treatment. We will validate the function of Ago TRIBE by RNA seq and utilize this tool to identify targets regulated by our screen hits. This work will help us understand miRNA-related mechanisms in angiosarcoma development.

Basic Science ***Graduate Student***

POSTER #42

IDENTIFYING THE METHYLATION MOTIF AND SUBSTRATE SELECTIVITY OF SETMAR

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Dysregulation of epigenetic regulators, including lysine methyltransferases (KMTs), is a hallmark of cancer. KMTs catalyze methylation of both histone and non-histone proteins, regulating protein function in a variety of ways. Identifying KMT substrates is critical to deciphering the role of KMTs in cancer phenotypes. Recent studies show the lysine methyltransferase fusion protein, SETMAR (also known as Metnase), is implicated in many cancer types. SETMAR has multiple splice variants, including one that lacks KMT activity (SETMAR-VarA). In bladder cancer, among others, changes in the levels of methyltransferase active and inactive SETMAR isoforms correlate with disease severity. To characterize the substrate selectivity of SETMAR, we performed in vitro methyltransferase assays using a peptide spot array with amino acid substitutions surrounding each position of the previously reported SETMAR substrate snRNP70 Lys-130. We discovered that SETMAR requires a tyrosine at the P+6 position relative to the methylated lysine. To elucidate the differences between the interactomes of full-length SETMAR and SETMAR-VarA, we performed proximity-based labeling combined with mass spectrometry-based proteomics. Several proteins that interacted with full-length SETMAR contained sequence motifs consistent with the preferred SETMAR methylation motif. Subsequent analysis of SETMAR methylation of these substrates revealed novel SETMAR substrates that we are currently investigating, including the ubiquitin ligase BRCC3 and the transcription complex CCR4-NOT. Our data provide detailed insight into the substrate selectivity of SETMAR and will help guide future studies to elucidate the role of SETMAR isoforms in cancer.

Basic Science ***Graduate Student***

POSTER #43

ELUCIDATING THE ROLE OF YBX1 IN ERG-POSITIVE PROSTATE CANCER

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Prostate cancer is the most common cancer in men, accounting for approximately 26% of male cancers. The ETS transcription factor ERG is fused to the androgen responsive promoter *TMPRSS2* and aberrantly expressed in 50% of prostate cancers. ERG interacts with a co-activator complex to drive prostate cancer, which includes the proteins EWS and PABPC1. Preliminary data suggests that an additional component of this complex is YBX1, a cold-shock transcription factor normally involved in proliferation and development. YBX1 has been implicated in multiple cancers and is upregulated in androgen-independent prostate cancer; however, its role in the ERG-EWS-PABPC1 complex is unclear. Previous research shows that YBX1 localizes to the nucleus in other cancers, so I predict that it plays a role in the nuclear localization of the ERG-EWS complex and maintains their interaction. I aim to evaluate the expression and localization of YBX1 in normal prostate and prostate cancer cell lines and to ascertain the requirement of YBX1 for oncogenic ERG function. I will immunoblot for YBX1 in a nuclear/cytoplasmic fraction of normal prostate and prostate cancer cell lines with and without ERG expression to identify the localization of YBX1. To determine the requirement for YBX1 in localization and maintenance of the ERG-EWS complex, I will perform overexpression and knockdown of YBX1 in the cell lines and perform pulldowns with and without YBX1 knockdown. YBX1 has its own DNA binding domain, so I will also perform ChIP-seq to see if YBX1 co-localizes with ERG, and if it alters where ERG binds. This research will improve our understanding of the molecular mechanism behind ERG-regulated prostate cancer and may distinguish YBX1 as a target for prostate cancer therapeutics.

Basic Science ***Graduate Student***

POSTER #44

THE TRITERPENOID CDDO-METHYL ESTER REQUIRES NRF2 TO DECREASE LUNG TUMOR BURDEN AND TO PROTECT AGAINST THE TOXICITY OF CHEMOTHERAPY IN EXPERIMENTAL LUNG CANCER

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The NRF2 cytoprotective pathway, a drug target for many inflammation-related diseases, regulates immune cell function. While the anti-inflammatory nature of NRF2 activation protects healthy cells from malignant transformation, cancer cells can utilize the pathway to promote resistance to anti-cancer drugs. Up to 30% of human lung adenocarcinomas acquire mutations in either *NFE2L2* or its negative regulator *KEAP1* which result in constitutive activation of the NRF2 pathway. However, despite our knowledge of the important immunomodulatory effects of NRF2, these effects are not well characterized in the context of cancer. With NRF2 activators now approved for clinical use, it is critical to understand the impact of these drugs in cancer. Triterpenoids including CDDO-methyl ester (CDDO-Me, also known as bardoxolone methyl) are potent pharmacological NRF2 activators with demonstrated anti-cancer activity in preclinical models. In an early-stage preclinical model of lung cancer, CDDO-Me significantly decreased tumor burden in a dose- and Nrf2-dependent manner and improved immune signatures within the tumor microenvironment. However, most human lung cancers are not diagnosed until more advanced stages. To test CDDO-Me in an established tumor intervention model, lung tumors were initiated with vinyl carbamate in A/J WT and Nrf2 knockout (KO) mice. Tumors were allowed to develop for 8 weeks post initiation, after which mice were fed either a vehicle control diet or CDDO-Me (50-100 mg/kg of diet) for an additional 8-12 weeks, alone or in combination with carboplatin and paclitaxel (C/P) at 50 mg/kg and 15 mg/kg, respectively, by IP injection every other week. CDDO-Me significantly ($p < 0.05$) decreased surface lung tumor counts 35-71% in a Nrf2-dependent manner, and C/P significantly ($p < 0.001$) reduced tumor burden 53-59% regardless of Nrf2 status. The combination of CDDO-Me + C/P reduced tumor burden in WT lungs by 84% ($p < 0.05$), more than either agent alone. Nrf2 KO mice had an approximately two-fold increase ($p < 0.001$) of surface tumors compared to WT mice in both studies, regardless of treatment. C/P reduced tumor burden similarly in Nrf2 WT and Nrf2 KO mice (53% and 59% reduction in tumor count, respectively). Interestingly, the combination of CDDO-Me + C/P increased T cell infiltration into the lungs of WT mice, but this change was not observed in groups treated with either agent alone. Importantly, CDDO-Me did not decrease the efficacy of C/P but protected WT mice from mortality and weight loss and lowered white and red blood cell counts. These studies suggest that NRF2 activation in advanced lung cancers can still decrease tumor burden by favorably modulating the immune microenvironment and, importantly, complements the anti-tumor efficacy of conventional chemotherapy while decreasing drug toxicity.

Basic Science ***Graduate Student***

POSTER #45

CHARACTERIZATION OF THE PRC2-REGULATED PROTEIN ZNF423 IN NF1-RELATED MALIGNANT PERIPHERAL NERVE SHEATH TUMORS

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Background: Malignant peripheral nerve sheath tumors (MPNST) are the most common and lethal malignant tumors in patients with Neurofibromatosis type 1 (NF1), and are characterized by recurrent biallelic inactivation of *NF1, CDKN2A/B* and PRC2 components *EED* or *SUZ12*. Surgical resection of MPNSTs is challenging and rarely possible as tumors often infiltrate vital nerves, and there are currently no approved targeted therapies for MPNST. Hence, the goal of this project is to identify potential therapeutic targets for NF1-related MPNST by probing the molecular drivers and mechanisms underlying MPNST cellular lineage, identity, and proliferation.

Methods: Human MPNST cell lines (control and PRC2-restored) and murine tumor cells-of-origin (*Nf1*$^{-/-}$ and *Nf1;Arf*$^{-/-}$) models were employed for RNA sequencing, immunoblotting, RNA interference, proteomic analysis of the kinome and cell-based assays.

Results: To investigate the transcriptional changes that occur as a result of PRC2 loss, we restored *SUZ12* in two PRC2-deficient MPNST lines. RNA sequencing revealed fourteen common transcription factors downregulated by PRC2 reconstitution. Amongst those downregulated was *ZNF423,* a transcription factor expressed in numerous immature cell populations, including neuronal and olfactory precursors, where it functions as a lineage-specific transcription factor by regulating differentiation-promoting functions. To validate these results, we utilized genetically engineered mouse models of benign plexiform neurofibroma and MPNST to isolate tumor cells-of-origin known as DNSCs (DRG/nerve root neurosphere cells). By RNA sequencing, we observed that 7 of 14 common transcription factors affected by PRC2 status were upregulated in murine cells. Upon *Nf1* inactivation, which occurs during PNF development, *Zfp423* (orthologous to human *ZNF423*) was repressed compared to wild type. However, when both *Nf1* and *Arf* were inactivated, expression was significantly higher than wild type. Ablation of *Suz12* by CRISPR/Cas9 to disrupt PRC2 function did not further elevate *Zfp423* transcription. We are currently testing whether reintroduction of ectopic SUZ12 will drive repression of *Zfp423*. Preliminary analysis of RNA sequencing following depletion by siRNA in human NF MPNST cell lines reveals that ZNF423 regulates key neuronal differentiation programs and may contribute to MPNST signatures of dedifferentiation.

Conclusions: ZNF423 depletion significantly reduces MPNST cell viability and proliferation suggesting that its disruption could interfere with tumor growth. Ongoing studies will further delineate ZNF423-dependent signaling pathways in human and murine MPNST models using omics approaches, cell-based phenotypic assays, and *in vivo* studies.

Basic Science ***Graduate Student***

POSTER #46

KEAP1 MUTATION IN LUNG CANCER CELLS LEADS TO INCREASED IMMUNOSUPPRESSIVE CAPABILITY

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Considerable advances have been made in lung cancer therapies, but there is still an unmet clinical need to improve survival for lung cancer patients. Immunotherapies have improved survival, although only 20-30% of patients respond to these treatments. Interestingly, cancers with mutations in *KEAP1*, the negative regulator of the NRF2 cytoprotective pathway, are resistant to immune checkpoint inhibition and correlate with decreased immune cell infiltration. NRF2 is known for promoting an anti-inflammatory phenotype when activated in immune cells, but the study of NRF2 activation in cancer cells has not been adequately assessed. The overall objective of this study is to determine how lung cancer cells with constitutive NRF2 activity interact with the immune microenvironment to promote cancer progression and metastasis. To assess this, we generated CRISPR-edited mouse lung cancer cell lines (Lewis Lung Carcinoma/LL2) with knockouts (KO) of the *KEAP1* and *NFE2L2* genes. The transcriptomic profiles of 3 *KEAP1* KO and 1 *NRF2* KO cell lines were compared to the parental wildtype (WT) line using RNA-sequencing. On unsupervised hierarchical clustering of differentially expressed genes, the 3 *KEAP1* KO cell lines clustered together and the *NRF2* KO clustered with WT cells. Importantly, canonical NRF2 pathway-related genes were significantly increased in *KEAP1* KO clones, but the same genes were either decreased or similar to WT in the *NRF2* KO clone. We then used flow cytometry to evaluate immunosuppressive surface markers known to suppress T cell function when expressed on cancer cells. Importantly, PD-L1, CD155, CD80, and CD86 were increased in the *KEAP1* KO lines compared to WT, but not in the *NRF2* KO clone. In addition, the cytokine release profile was differentially regulated in the *KEAP1* KO clones in a manner that could alter T cell recruitment and function. These results were also consistent with gene expression in the sequencing dataset. Finally, using an orthotopic allograft model, we completed a preliminary immunophenotyping study of lung tumors for WT, *KEAP1* KO, and *NRF2* KO cell lines. The activation status of $CD8^+$ T cells and the ratio of $CD8^+/CD4^+CD25^+$ T cells (a prognostic marker) were decreased in *KEAP1* KO tumors. Taken together, these data suggest cancers with *KEAP1* mutations may increase tumor growth through suppression of T cells.

Basic Science ***Graduate Student***

POSTER #47

EGFR ACTIVATION BY PP2A DRIVES PRO-TUMORIGENIC PHENOTYPES IN PANCREATIC CANCER

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Pancreatic ductal adenocarcinoma (PDAC) stands to become the second most deadly cancer by 2030. The small GTPase, KRAS, is mutated in over 90% of PDAC patients and considered the primary driver mutation. Despite being an almost ubiquitous event, KRAS mutations have been difficult to target therapeutically, particularly $KRAS^{G12D}$, the most common mutation in PDAC. In addition to these pharmacological challenges, KRAS mutations have been shown to drive signaling plasticity and therapeutic resistance through phosphorylation cascades in most cancers. Protein phosphatases are master regulators of kinase signaling, however the contribution of phosphatase deregulation to mutant KRAS cancer phenotypes is poorly understood. Protein phosphatase 2A (PP2A) inhibits effectors downstream of KRAS, placing this family of enzymes as key regulators of PDAC oncogenic signaling. However, previous studies utilizing small molecule activating compounds of PP2A show a heterogeneous response in PDAC, with some cell lines displaying increased oncogenic signaling despite induction of phosphatase activity. Similarly, specific PP2A subunits have shown to play pro-tumorigenic roles in one tissue type and tumor suppressive roles in others. This suggests that there are unique PP2A signaling cascades that occur in PDAC which have yet to be elucidated. To determine the impact of PP2A activation on oncogenic feedback loops, PP2A-B56a knockout and overexpression studies as well as pharmacological activation of PP2A-B56a in human PDAC cell lines evaluated changes in oncogenic signaling and phenotypes. Activation of PP2A-B56a leads to the suppression of specific oncogenic pathways, however, this is accompanied with an increase in oncogenic phenotypes and activation of the epidermal growth factor receptor (EGFR). EGFR is a critical signaling node in PDAC as inhibition or loss of EGFR prevents KRAS-driven tumorigenesis and increased EGFR activity is associated with poor patient outcome. This activation of EGFR by PP2A-B56a is through increased expression and processing of EGFR ligands, specifically amphiregulin, HB-EGF, and epiregulin. The activation of EGFR signaling drives tumorigenic phenotypes and reduces the anti-tumorigenic effect of PP2A activity. The addition of an EGFR inhibitor to PP2A activation blocks this aberrant signaling and results in synergistic cell death. Together, these studies implicate a previously undescribed role for PP2A-B56a in EGFR signaling in PDAC.

Basic Science ***Graduate Student***

POSTER #48

ROLE OF RAN REGULATION ON CENTROSOME CLUSTERING BY KINESIN-14 MOLECULAR MOTORS

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Approximately 80% breast cancer cases exhibit centrosome amplification, which is the presence of more than two centrosomes in a cell. Cancer cells with centrosome amplification cluster their extra centrosomes into two poles and undergo a bipolar division, which results in aneuploidy leading to genome instability. Centrosome clustering in cancer cells is facilitated by Kinesin-14 (K-14), a molecular motor, which is amplified in 30% of breast cancers. K-14 has a motor domain that walks towards microtubule minus ends and a tail that crosslinks and slides both parallel and anti-parallel microtubules. K-14 is a spindle assembly factor (SAF) that is regulated by the Ran-GTP gradient. High Ran-GTP levels near chromatin promote spindle assembly by releasing SAFs from importin-mediated inhibition. K-14 mediated anti-parallel MT cross-linking is inhibited by importins and is Ran-regulated. Ran levels are higher in cancer cells with increased genomic content, and high Ran recruits more K-14 to the spindle, which may help promote centrosome clustering. The K-14 tail contains two microtubule binding domains (MBD1 and MBD2), and MBD1 contains the nuclear localization signal (NLS) that binds importins. Knockdown/rescue experiments revealed that these two MBDs are involved in different functions: MBD1 regulated pole clustering, and MBD2 regulated spindle length. Because pole clustering is dependent on MBD1, we analyzed cancer patient mutations in MBD1 and identified two mutations within the NLS. These mutations reduced the interaction with importins but did not dramatically reduce MT binding, indicating that they may be useful to dissect Ran-regulation from MBD1-dependent MT cross-linking. To test if pole clustering was regulated by the MT binding activity of MBD1 or by the Ran-regulation of the NLS within MBD1, the K-14 mutants were expressed in cells treated with paclitaxel to induce spindle multipolarity. The ability of the mutants to rescue multipolarity was not different from that of wild-type K-14, suggesting that it is not the importin affinity, but the MT binding by MBD1 that is critical for centrosome clustering. In addition, the presence of chromatin between the extra centrosomes in multipolar spindles, and thus high Ran-GTP levels, may facilitate MBD1 mediated anti-parallel crosslinking and sliding of MTs in this region. To determine how Ran regulates other spindle functions of K-14, analogous mutations were generated in *Xenopus* K-14, as *Xenopus* egg extracts are an ideal *in vitro* system to study Ran-regulated spindle assembly and organization. The mutations in the *Xenopus* K-14 tail showed reduced importin affinity without a drastic impact on MT binding, suggesting that we have identified separation of function mutations that will be important for dissecting Ran regulation of SAFs. Our findings may provide us with a tool to identify small molecule inhibitors targeting the MBD1 of K-14 to specifically inhibit its clustering ability and thereby kill cancer cells.

Basic Science ***Graduate Student***

POSTER #49

ROLE OF TRYPTOPHAN METABOLIZING ENZYMES IN MULTIPLE MYELOMA SURVIVAL AND IMMUNE EVASION

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Introduction:

Multiple myeloma (MM) is a hematological malignancy caused by abnormally proliferating plasma cells in the bone marrow. It is considered incurable. Recently new immunotherapies have been developed for MM, opening new avenues for treatment, but even with immunotherapy MM patients continue to relapse and perish from MM. Our lab has previously found that MM interacts with Dendritic Cells (DCs) in the Bone Marrow Microenvironment (BMME) and that this interaction leads to the production of the immunosuppressive enzyme Indoleamine 2,3-dioxygenase 1(IDO1) by DCs. IDO1 catabolizes tryptophan(TRP) to kynurenine(KYN). The depletion of TRP in the BMME has been known to suppress T-effector activity, while the KYN activates the transcription factor Aryl Hydrocarbon Receptor(AHR), inducing a pro-survival signal in MM and Tregs. Understanding how MM is able to evade the immune system and support its survival could lead to novel treatment targets.

Methods:

Patient RNA expression data were taken from the CoMMpass database. MM cell lines U266, 8226, MM1S and KMS11 were measured for expression of TRP□KYN metabolizing enzymes through western blot and qPCR. TDO2 was inhibited in cell lines with inhibitor 680C91 or knocked down using shRNA. Activation of AHR was measured through expression of CYP1A1, a transcriptional downstream target. CYP1A1 expression was measured through qPCR. For some experiments MM cell lines were co-cultured with monocyte-derived DC. Cell viability was measured by flow cytometry. KYN production was measured through ELISA.

Results:

We have found that MM cells produce KYN in the absence of DC, indicating that metabolize TRP independently. MM cells don't express IDO1, but they do express Tryptophan Dioxygenase 2(TDO2), which metabolizes TRP->KYN like IDO1. TDO2 expression has been noted in several cancer types, but its role in MM has not been previously described. Cell lines 8226, MM1S and KMS11 express TDO2, while U266 does not. Patients in the highest quartile of TDO2 expression had significantly worse progression-free survival and overall survival outcomes. Pharmacological inhibition and shRNA knockdown of TDO2 led to decreased cell survival and proliferation. Inhibition of TDO2 led to decreased expression of both CYP1A1 and AHR, indicating that it prevented AHR activation.

Discussion

We have found that MM cells express tryptophan metabolizing enzyme TDO2, indicating that they can metabolize TRP->KYN independently from interaction with DCs. The depletion of TRP and creation of KYN could repress T-effector activity and increase Treg activity, while supporting MM survival through the activation of AHR. As MM cells can create TRP metabolizing enzymes without BMME interaction, this indicates that they could create an immunosuppressive and pro-MM survival microenvironment even once

they escape the BMME. Studying the production of TDO2 in MM could lead to novel treatment targets that would reduce MM survival and make MM more sensitive to immune response.

Basic Science ***Graduate Student***

POSTER #50

IB-DNQ AND RUCAPARIB DUAL TREATMENT ALTERS CELL CYCLE REGULATION AND DNA REPAIR IN TRIPLE NEGATIVE BREAST CANCER CELLS

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Background:Triple negative breast cancer (TNBC), characterized by the lack of three canonical receptors, is unresponsive to commonly used hormonal therapies. One potential TNBC-specific therapeutic target is NQO1, as it is highly expressed in many TNBC patients and lowly expressed in non-cancer tissues. DNA damage induced by NQO1 bioactivatable drugs in combination with Rucaparib-mediated inhibition of PARP1-dependent DNA repair synergistically induces cell death.

Methods:To gain a better understanding of the mechanisms behind this synergistic effect, we used global proteomics, phosphoproteomics, and thermal proteome profiling to analyze changes in protein abundance, phosphorylation and protein thermal stability.

Results:Very few protein abundance changes resulted from single or dual agent treatment; however, protein phosphorylation and thermal stability were impacted. Histone H2AX was among several proteins identified to have increased phosphorylation when cells were treated with the combination of IB-DNQ and Rucaparib, validating that the drugs induced persistent DNA damage. Thermal proteome profiling revealed destabilization of H2AX following combination treatment, potentially a result of the increase in phosphorylation. Kinase substrate enrichment analysis predicted altered activity for kinases involved in DNA repair and cell cycle following dual agent treatment. Further biophysical analysis of these two processes revealed alterations in SWI/SNF complex association and tubulin/p53 interactions.

Conclusions:Our findings that the drugs target DNA repair and cell cycle regulation, canonical cancer treatment targets, in a way that is dependent on increased expression of a protein selectively found to be upregulated in cancers without impacting protein abundance illustrate that multi-omics methodologies are important to gain a deeper understanding of the mechanisms behind treatment induced cancer cell death.

Basic Science ***Graduate Student***

POSTER #51

INVESTIGATING THE KINASE-INDEPENDENT AUTOREGULATORY LOOP OF PIM2 IN MULTIPLE MYELOMA

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Background: PIM2, an oncogenic serine/threonine kinase, is overexpressed in multiple myeloma (MM) and associated with poor prognosis. We hypothesize that PIM2 may have a previously unrecognized kinase-independent (Ki) function that regulates its own expression and promotes MM cell survival through an autoregulatory loop involving the transcription factor MYC.

Methods: To investigate the potential mechanism of PIM2 Ki autoregulation in MM cell lines, we utilized luciferase reporter assays, chromatin immunoprecipitation (ChIP), and pharmacological inhibition. Luciferase assays were performed using the full-length PIM2 promoter to assess transcriptional activity in response to PIM2 Ki inhibitors (JP1 and JP2) and a PIM2 kinase-dependent (Kdep) inhibitor (AZD1208). ChIP-qPCR was employed to evaluate MYC occupancy at the PIM2 promoter under different PIM2 inhibitor treatments.

Results: Luciferase assays revealed a significant decrease in PIM2 promoter activity upon treatment with the PIM2 Ki inhibitor JP1, but not with the Kdep inhibitor AZD1208. ChIP-qPCR demonstrated enrichment of MYC at the PIM2 promoter, which was disrupted by JP1 treatment and increased by AZD1208 treatment. Furthermore, pharmacological inhibition of MYC using the small molecule inhibitor 10058-F4 resulted in decreased PIM2 protein levels, similar to the effects of JP1 and JP2. These findings suggest that MYC may directly bind to the PIM2 promoter and facilitate a PIM2 Ki autoregulatory loop that maintains aberrant PIM2 overexpression in MM cells.

Conclusions: Our preliminary data support the hypothesis of a novel PIM2 kinase-independent autoregulatory mechanism involving MYC in MM. Disruption of this potential PIM2 Ki autoregulatory loop using selective inhibitors (JP1 and JP2) decreases PIM2 expression and promoter activity, suggesting it as a potential therapeutic target. Further investigation into the specific cis-regulatory elements and binding sites governing PIM2 Ki autoregulation may uncover new vulnerabilities to exploit in MM treatment.

Basic Science ***Graduate Student***

POSTER #52

EFFECTS OF PROCR+/ ZEB1+/ PDGFRα+ FIBROBLASTS ON BREAST TUMOR MICROENVIRONMENT

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The women of African ancestry have been shown to develop more aggressive breast cancer with worse prognosis than women of European ancestry. Most of these differences were attributed to disparity in health care access. However, even after considering health disparity and socioeconomic status, this trend continues. In women of African ancestry, it has also been shown that despite having high number of tumor infiltrating lymphocytes (TILs), their prognosis is still worse than that of women of European ancestry. These observations suggest that TILs in breast tumors of women of African ancestry are non-functional. Mechanisms behind this differential TIL activity are unknown but could involve genetic ancestry-dependent variability in the normal breast biology.

To address the influence of genetic ancestry on T cell function in the breast tumor environment, we are studying a population of mesenchymal stem-like cells with fibroblast like properties expressing PROCR, ZEB1 and PDGFRα (PZP cells), which are enriched in the breast tissue of healthy women of African ancestry. These cells can trans-differentiate into adipogenic and osteogenic lineages under appropriate differentiation cocktails. Interaction of PZP cells with epithelial cells also upregulates IL6 and TAGLN expression in PZP cells, which could potentially affect the tumor microenvironment. Our preliminary data suggest multi-directional interaction of PZP cell with surrounding cells leading to altered breast microenvironment. Interaction of PZP cells with luminal breast epithelial cells leads to luminal epithelial cells acquiring basal cell characteristics. Secretory factors from PZP cells attenuate interferon gamma production by CD8 T lymphocytes while promoting their proliferation. From these data, we hypothesize that PZP cells modify the tumor microenvironment to favor tumor growth through the interactions with epithelial and immune cells. Therefore, PZP cell-derived factors could be targets for therapeutic interventions.

To study how this occurs, we aim to investigate the effect of PZP cells on epithelial cells and immune cells. In the first aim, we will investigate the effects of secretory factors from parental, adipogenic-differentiated, osteogenic-differentiated PZP cells on primary, immortalized and transformed breast epithelial cells with respect to stemness, luminal-basal hybrid phenotype and sensitivity to select drugs. The second aim will study the effect of secretory factors from PZP cells, PZP:epithelial co-culture, PZP differentiated adipocytes on CD8 T cell function.

The tumor microenvironment is a complex system which affects the growth of the tumor from the inside. This system is made more complex by the presence of PZP cells in the breast tissue of women of African ancestry. A positive outcome from the study will help to design therapies that considers genetic ancestry as one of the factors that impacts outcome.

Basic Science ***Graduate Student***

POSTER #53

COORDINATION BETWEEN GCN2 EIF2 KINASE AND P53 SIGNALING SUPPORTS PURINE METABOLISM AND PROGRESSION OF PROSTATE CANCER

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The Integrated Stress Response (ISR) features four protein kinases (GCN2, PERK, HRI, PKR) that each sense distinct cellular stresses, phosphorylating eIF2 to direct gene expression that serves to mitigate cell damage and restore homeostasis. Previously, we demonstrated that GCN2 and the ISR are constitutively active in prostate cancer (PCa) and required to maintain amino acid (AA) homeostasis to sustain tumor growth. GCN2 is a sensor of AA availability, and basal GCN2 activation in PCa results in the enhanced expression of transporters that import AAs that are required for proliferation. However, while genetic or pharmacological inhibition of GCN2 reduces growth of PCa cells in culture and in mice, minimal cell death is observed. We hypothesized that other compensatory stress response pathways may be induced in GCN2-deficient PCa cells that promote cell survival.

Here, we used androgen-sensitive and castration-resistant human PCa cell lines, organoid cultures, and mouse xenograft models, along with transcriptomic and metabolomic analyses, and determined that loss of GCN2 in PCa cells activates a secondary stress pathway involving the tumor suppressor p53. The transcription factor p53 is activated by diverse stress conditions and regulates the expression of genes involved in cell-cycle control, DNA damage response, senescence, and apoptosis. We found that induction of p53 following GCN2 inhibition in PCa promotes a quiescent phenotype featuring cell-cycle arrest and senescence. We discovered that GCN2 is also central for maintenance of purines in PCa and their depletion in response to loss of GCN2 triggers impaired ribosome biogenesis.

Interestingly, we also found that loss of p53 resulted in increased activation of GCN2, suggesting that p53 may also facilitate AA homeostasis in PCa cells. Indeed, p53 regulates AA levels in PCa cells, and increased activation of GCN2 in p53-deficient cells was diminished by supplementation with exogenous AAs. Loss of p53 resulted in reduced proliferation and enhanced cell death in GCN2-deficient cells in organoid and xenograft PCa models. Highlighting the importance of GCN2 in the manintenance of nucleotide metabolism in PCa, we discovered that targeted inhibition of *de novo* purine biosynthesis results in the selective death of GCN2-deficient cells. Treatment of xenograft tumors or organoids with an inhibitor of GART, an enzyme necessary for *de novo* purine synthesis, resulted in selective death of GCN2-deficient PCa cells. These results suggest that p53 facilitates cell survival in GCN2-deficient PCa cells and that purine metabolism is a metabolic vulnerability in GCN2-deficient PCa cells that can be exploited therapeutically. Our study provides insights into how the ISR is coordinated with other adaptive stress response pathways to enhance PCa proliferation and survival, and provides new insights into combination therapeutics for the treatment of PCa.

Basic Science ***Graduate Student***

POSTER #54

ENGINEERED SUPPRESSOR TRNAS RESCUES NONSENSE MUTATIONS IN TP53

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Background:

Over 10% of genetic diseases are caused by mutations that introduce stop codons, known as **premature termination codons** (PTCs), into protein coding genes. mRNA transcripts harboring PTCs result in premature polypeptide cleavage from the ribosome which leads to loss of protein function. One promising new approach to address diseases that are caused by PTCs relies on the use of engineered suppressor transfer RNAs (sup-tRNAs), which are tRNAs that are modified to recognize one of the 3 stop codons. Sup-tRNAs can incorporate **amino acids** (AAs) at PTCs, outcompeting endogenous factors that cleave the polypeptide, and inducing stop codon readthrough. Use lipid nanoparticle or AAV delivery vehicles, sup-tRNAs were recently shown to rescue disease phenotypes in animal models of cystic fibrosis and muscular dystrophy; however, their application as anticancer therapeutics has not yet been explored. Tumor suppressor gene ***TP53***is one of the most mutated proteins in cancer—**11% of cancer-associated mutations** on *TP53* are PTCs. We hypothesize that sup-tRNAs can induce translational readthrough of PTCs in the *TP53* gene to restore p53 protein levels and inhibit cancer cell proliferation.

Methods:

We recently developed a novel class of sup-tRNAs that efficiently suppress all three stop codons (TGA, TAA, and TGA). Here, we tested the ability of these sup-tRNAs to elicit translational readthrough of PTCs in cancer cells harboring homozygous mutations in *TP53*.p53 protein levels were quantified by immunoblotting, while p53 activity was quantified with a combination of luciferase-based transcriptional assays and qRT-PCR.

Results:

Treatment with sup-tRNAs resulted in an increase in levels of p53 protein levels as determined by immunoblotting. Moreover, sup-tRNA-treated cells showed increased expression of several downstream genes that are transactivated by p53.

Conclusions:

Sup-tRNAs are able to restore functionally active p53 protein in cancer cells carrying PTC mutations in *TP53.* Sup-tRNAs are a potential approach to help drive tumor suppression. This **tRNA-based technology** is highly applicable for several other disease models; sup-tRNAs sequences are also significantly smaller compared to gene therapy transgenes. The use of **sup-tRNAs for gene therapy** can overcome current hurdles of transgene capacity. Further validating this therapy in animal models will help solidify its potential as a clinical therapeutic.

Basic Science ***Graduate Student***

POSTER #55

TARGETING BASAL MARKER 1 WITH CAR-T THERAPY: A NOVEL APPROACH FOR THE TREATMENT OF PROSTATE CANCER AND METASTATIC TRIPLE-NEGATIVE BREAST CANCER

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Prostate cancer (PCa) and metastatic triple-negative breast cancer (mTNBC) are two hard-to-treat malignancies, with resistance and unresponsiveness to current therapies. This study explores the potential of Basal Marker 1(BM1), a tumor-associated antigen upregulated in both basal-like PCa and mTNBC, as a target for chimeric antigen receptor T-cell (CAR-T) immunotherapy against PCa and mTNBC. We hypothesize that BM1-positive (BM1+) PCa and mTNBC can be eradicated by anti-BM1 CAR-T cells *in vitro* and *in vivo*. We assessed the efficacy of anti-BM1 CAR-T cells against PCa (DU145, DU145-BM1$^{-/-}$) and mTNBC (MDA-MB-468, MDA-MB-468-BM1$^{-/-}$) cell lines *in vitro* and *in vivo*. The anti-BM1 CAR-T cells demonstrated significant target-selective cytotoxicity against BM1+ DU145 and BM1+ MDA-MB-468 cell lines compared to their BM1$^{-/-}$ counterparts, establishing its selective cytotoxic potential *in vitro*. NOD SCID mice were engrafted with PCa and mTNBC cell lines and treated with CAR-T therapy in combination with irradiation and/or anti-PD-1 therapy. We anticipate that mice bearing BM1-high tumors will demonstrate extended survival or potential remission following anti-BM1 CAR-T treatment, which may be enhanced by irradiation and PD-1 blockade. Our study has demonstrated the potent target-cytotoxicity of anti-BM1 CAR-T therapy against PCa and mTNBC *in vitro*. We are currently investigating its efficacy *in vivo*, aiming to establish a novel immunotherapy for advanced PCa and mTNBC.

Basic Science ***Graduate Student***

POSTER #56

UNVEILING NEPC ORIGINS AND VULNERABILITIES THROUGH SINGLE-CELL ANALYSIS AND DRUG SCREENING

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Neuroendocrine prostate cancer (NEPC) is an aggressive, treatment-resistant subtype that often emerges after androgen deprivation therapy and treatment with novel androgen receptor (AR) pathway inhibitors. While recent studies suggest that luminal epithelial (LE) cells may transdifferentiate into neuroendocrine (NE) cells, the cellular events and tumor microenvironment factors driving this transition remain poorly understood. To address this knowledge gap, we integrated single-cell RNA-sequencing (scRNA-seq) analysis and high-throughput drug screening to elucidate the cellular origins and therapeutic vulnerabilities of NEPC.

Our integrative analysis of publicly available scRNA-seq datasets from prostate cancer patients revealed that NE cells engage in extensive communication with fibroblasts and immune cells, and that AR+ and AR- LE cells may give rise to distinct NE subpopulations. Furthermore, NE populations exhibit distinct metabolic patterns compared to LE cells and correlate with worse clinical outcomes.

To identify compounds that can exploit the lineage plasticity of NEPC cells, we performed a high-throughput screening for agents that induce neuroendocrine-to-luminal re-differentiation (NLrD) and re-establish AR signaling, potentially re-sensitizing NEPC cells to AR inhibitors like enzalutamide. We generated a stable NEPC reporter cell line, NEPCnd, with a dual-luciferase system, optimized the screening format, and employed the OCTAD computational tool to select 300 FDA-approved compounds predicted to reverse the NEPC gene signature. Primary screening will identify candidates that stimulate AR-responsive firefly luciferase expression dose-dependently with low nonspecific cytotoxicity, followed by validation of NLrD marker expression changes and combination assays with enzalutamide.

By integrating scRNA-seq analysis and drug screening, this study aims to uncover the cellular origins and microenvironment factors driving NEPC and develop a novel treatment strategy that exploits the lineage plasticity of prostate cancer cells to re-sensitize them to existing therapies.

Basic Science ***Graduate Student***

POSTER #57

STABILIZATION OF CHECKPOINT KINASE 1 BY THE LNCRNA HOTAIR PROMOTES PARP INHIBITOR RESISTANCE IN HOMOLOGOUS RECOMBINATION PROFICIENT HIGH GRADE SEROUS OVARIAN CANCER

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Poly (ADP-ribose) polymerase (PARP) inhibitors (PARPis) are an important treatment modality for homologous recombination deficient (HRD) cancers, including high grade serous ovarian cancer (HGSOC), breast, pancreatic and prostate cancers. PARP is an enzyme that mediates DNA single-strand break (SSB). PARPis trap PARP at the site of SSB and prevent DNA SSB repair, which eventually leads to double-strand DNA breaks (DSBs). DSBs are normally repaired through the HR pathway, and PARPis are highly efficient in the context of HRD disease. However, the majority of HGSOC are HR proficient (HRP), and the use of PARPis for patients with HRP tumors represents an unmet therapeutic need. The long non-coding RNA (lncRNA) HOX transcript antisense RNA (HOTAIR) is frequently over-expressed in HGSOC and contributes to chemoresistance. We examined the role of HOTAIR in the response to PARPis in HRP HGSOC cell lines. Paired CRISPR guide RNAs were used to delete the functional sites of HOTAIR in OVCAR3 and produce a loss-of-function HOTAIR phenotype. Knockout of HOTAIR or siRNA knockdown of HOTAIR sensitized ($p<0.05$) HGSOC cell lines to PARPis olaparib and talazoparib. HOTAIR upregulated the expression of checkpoint kinase 1 (CHK1), a key kinase involved in DNA damage response. While talazoparib treatment increased ($p<0.05$) expression of CHK1, HOTAIR KO decreased ($p<0.05$) CHK1 activation by PARPi. In contrast, overexpression of HOTAIR upregulated ($p<0.05$) talazoparib-induced activation of CHK1. It was reported that CHK1 inhibition converted PARPi-insensitive HRP cells to PARPi-sensitive cells. Thus, we hypothesized that overexpression of HOTAIR contributes to PARPi resistance in HGSOC cell lines by regulating CHK1. Furthermore, the DNA repair protein RAD51, which interacts with CHK1 in response to DNA damage, was reported to be necessary to promote the reversal of stalled replication forks and protect the reversed fork from degradation. We demonstrated that RAD51 expression was reduced ($p<0.05$) in HOTAIR KO cells treated with talazoparib, while overexpression of HOTAIR increased ($p<0.05$) talazoparib-induced activation of RAD51. In addition to a role in replication stress, CHK1 and RAD51 contribute to DNA damage repair through the HR repair pathway. To further examine the role of HOTAIR in DNA damage repair, we generated HOTAIR overexpressing HGSOC cells and examined the response to PARPi treatment by using the comet assay to assess DNA damage. PARPi-induced DNA damage was reduced ($p<0.001$), based on comet tail length. Collectively, these findings suggest that HOTAIR functions by regulating the role of CHK1 in replication fork protection and DNA damage repair in HGSOC and in turn contributes to PARPi resistance in HRP tumors.

Basic Science ***Graduate Student***

POSTER #58

THE COMBINED INHIBITION OF SREBP AND MTORC1 SIGNALING SYNERGISTICALLY INHIBITS THE PROLIFERATION OF B CELL LYMPHOMA

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Sterol regulatory element-binding protein (SREBP) signaling plays a crucial role in maintaining sterol homeostasis during B cell activation and the proliferation of germinal center B cells. It is unclear whether this pathway can be targeted to effectively treat B cell lymphoma. We discovered that inhibiting SREBP signaling or its downstream target HMG-CoA reductase (HMGCR) using Fatostatin or Simvastatin effectively restrains the proliferation of B cell lymphoma cells. However, B cell lymphoma cells activate the mTORC1-pS6 pathway in response to statin treatment, suggesting a possible mechanism to counteract statin-induced cell cycle arrest. Combining low dose statin treatment with the mTOR inhibitor rapamycin demonstrates a synergistic effect in inhibiting B cell lymphoma proliferation, cell cycle progression and lipid raft generation. These findings emphasize the potential of a combined therapy approach targeting both SREBP and mTORC1 as a novel treatment strategy for B cell lymphoma.

Basic Science ***Graduate Student***

POSTER #59

SETX BINDS TO THE HPV E2 PROTEIN TO RESOLVE R-LOOPS ON THE VIRAL GENOME

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Human papillomaviruses (HPV) are DNA tumor viruses that infects cutaneous and mucosal epithelia where high-risk (HR) HPV infections can lead to cervical, oropharyngeal, and anogenital cancers. The viral E1 and E2 proteins are essential for genome replication. HPV E2 recruits the helicase E1 to the origin of replication (ori) to initial replication. R-loops are RNA-DNA hybrids that form during transcription, DNA replication and repair, to displace single-stranded DNA. These DNA:RNA hybrids regulate gene expression and chromatin structure. Senataxin (SETX) is an RNA:DNA helicase enzyme that facilitates the initiation of the unwinding process of the RNA/DNA hybrid. The physiological functions of SETX range from gene expression to the maintenance of genome integrity. R-loops are formed during HPV replication and genome transcription. Depletion of SETX using siRNAs increased the presence of R-loops at the viral early promoter in HPV-31 (CIN612) and HPV-16 (W12) episomal HPV cell lines. We detected E2 and SETX at the viral promoter. We hypothesized that E2 may recruit SETX to the genome to resolve R-loops. We observed that SETX co-immuoprecipatates with HPV-31 and HPV-16 E2. These results imply that E2 associates with SETX to prevent accumulation of R-loops in cells harboring episomal HPV.

Basic Science ***IADEP Student (post-bac)***

POSTER #60

EVALUATING THE ROLE OF T CELLS IN NF1-PERIPHERAL NERVE SHEATH TUMOR (PNST) PROGRESSION

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Neurofibromatosis type 1 (NF1) is a cancer predisposition syndrome caused by a mutation in the *NF1* tumor suppressor gene that occurs in 1:3000 live births. Loss of neurofibromin, a GTPase activating protein for p21 RAS, causes hyperactivation of the RAS pathway that leads to the formation of plexiform neurofibromas (PNF) in up to 50% of individuals with NF1. In the majority of NF1 patients, PNF grow slowly, typically reaching stability in adulthood. However, a subpopulation of NF1 patients experience progression to atypical neurofibromatous neoplasm of uncertain biologic potential (ANNUBP) and transform to malignant peripheral nerve sheath tumor (MPNST). MPNSTs are the leading cause of premature death in NF1 patients with a 5-year survival rate of 20-50%. Therefore, understanding the molecular and cellular interactions that mediate progression along the peripheral nerve sheath tumor (PNST) continuum is critical to improving NF1 patient survival. The genetic events governing neurofibroma progression and malignant transformation have been extensively studied and several key driver events have been implicated. However, preclinical and natural history studies suggest that these genetic changes may not fully account for the clinical heterogeneity of PNF and ANNUBP precursor lesions and their evolution to MPNST. Therefore, we postulate that additional factors either intrinsic to neoplastic Schwann cell progenitors or within the tumor microenvironment play a critical role in modulating the growth and malignant potential of neurofibroma. Our recent work defining the spatial gene expression profile of human NF1-associated tumors identified alterations in signatures of immune surveillance, including T cell activation and cytotoxicity, across the PNST continuum. Anti-tumor T cell response signatures were upregulated in ANNUBP, whereas precursor lesions that progressed to MPNST were characterized by signatures of T cell exhaustion. Furthermore, immunofluorescence staining showed that human ANNUBPs were characterized by increased infiltration of $CD4^{+}FOXP3^{-}$ and $CD8^{+}FOXP3^{-}$ T cells, while $FOXP3^{+}$ T cells (T regulatory cells) predominated in MPNST. While the density and functional states of T cells infiltrating the tumor microenvironment are important predictors of therapeutic response and prognosis in many human cancers, the functional role of distinct T cell subsets in governing neurofibroma progression to MPNST remains undefined. The objective of this work is to define the T cell subsets present at various stages of the PNST continuum and investigate their functional role in tumor progression. We hypothesize that effector CD4 and/or cytotoxic CD8+ T cells are critical in preventing malignant transformation of PNF and ANNUBP precursors. Ultimately, these studies will advance our understanding of the functional role of T cell subsets across the PNST continuum and inform future diagnostic and therapeutic strategies for patients with NF1.

Basic Science ***MD/PhD Student***

POSTER #61

DEVELOPMENT OF A NOVEL HPV16 E6E7 MOUSE MODEL

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The Human Papilloma Virus (HPV) is a double stranded DNA tumor virus that infects cutaneous and mucosal epithelium. The viral E6 and E7 oncogenes are expressed in all HPV cancers. They produce viral oncoproteins that interfere with cell cycle regulation and DNA repair mechanisms. To study the entire lifecycle of the virus in vivo, the Mus musculus PV (MmuPV1) infection model is used. The MmuPV1 genome organization is similar to that of HPV. The MmuPV1 has been shown to infect, propagate, and induce neoplastic disease in the skin, cervix, anogenital tract and oropharynx of immunosuppressed mice. However, this model is limited because PVs are species specific and will not propagate in a host that is not its own. Our goal was to generate a MmuPV1 mouse model with the HPV-16 E6 and E7 genes. It is important to study and develop antivirals tothe human PV, not the mouse oncogenes. To do this, we replaced the MmuPV1 E6 and E7 with the HPV-16 E6 and E7 genes (MmuPV1 16E6E7). This genome was injected into the tails of nude mice to study wart development.

To study replication and transcription of the MmuPV1 16E6E7 genome, the L1 and L2 genes were deleted and replaced with a neomycin cassette to allow selection in a cell culture system. We harvested and isolated mouse keratinocytes from newborn mice, in which we infected the neomycin version of MmuPV1 16E6E7 genome (Mkert+chimera neo). These cells expressed HPV-16 E6 and E7 mRNA. These keratinocytes also expressed the HPV-16 E7 protein and p53 was downregulated, a marker for E6 function. Our data suggest that HPV-16 E6 and E7 are expressed in this chimera genomecontext.

Basic Science ***Post-Baccalaureate (IADEP)***

POSTER #62

MECHANISMS OF BET INHIBITOR-MEDIATED GROWTH SUPPRESSION IN NAïVE AND METASTATIC DISEASE MODELS OF OSTEOSARCOMA

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Osteosarcoma (OS) is an aggressive bone cancer in pediatric, adolescent, and young adult patients. Survival rate for metastatic and relapsed OS patients remains dismal at <30%. Additionally, no effective standardized salvage therapy currently exists for these patients, in part, due to genomic complexities arising from moderate levels of replication stress (RS), which is a hallmark of OS. Bromodomain and extra-terminal domain (BET) proteins (BRD2,3,4) are a family of epigenetic readers that not only regulate transcriptional networks, but also contribute to DNA replication and RS. While moderate RS levels cause genomic instability that contributes to OS progression, high levels of RS can induce cell death. Notably, BET inhibitors (BETi) create an imbalance between transcription-replication kinetics which, ultimately, leads to cell death from the exacerbated RS. Utilization of BETi to target oncogenic RS is an under-explored option for OS. Thus, we hypothesize that bivalent BETi (AZD5153) leads to decreased OS cell growth via gene dysregulation and increased DNA damage. Our in vivo studies demonstrate that, AZD5153 monotherapy significantly suppressed tumor growth in OS patient-derived xenografts (PDXs) derived from both naïve (PDX96) and metastatic (TT2) OS compared to vehicle ($p<0.05$). Decreased PDX96 growth correlated with increased γ-H2AX and increased expression of pro-apoptotic genes following AZD5153 exposure, indicative of increased RS and cell death. BETi (AZD5153, BMS-986378, ZEN-3694) are currently under investigation in clinical trials for pediatric and adult solid tumors as well as acute myeloid leukemia and lymphoma. However, little is known about BETi- induced molecular changes and adaptive responses in OS. Therefore, the objective of our current investigation is to gain further mechanistic insights into BETi-mediated growth inhibition and to prioritize a BETi for clinical trial development in OS and other sarcomas. In vitro BETi screening data of OS cell lines indicate that AZD5153 is the most potent BETi compared to BMS-986378 and ZEN-3694. Whether this translates into differences in vivo is an area of future study. Furthermore, RNA-seq analysis and protein validation from vehicle versus AZD5153-treated PDX96 highlighted dysregulation of several key genes involved in the DNA damage response including downregulation of TCF7, a downstream effector of the Wnt/ β-Catenin pathway. The abnormal activation of the Wnt/ β-Catenin pathway activates oncogenic programs promoting tumor progression and metastasis. Therefore, as a way to interrogate mechanism, we determined if this pathway is also downregulated in BETi-treated in-vitro OS cultures. Our in-vitro data suggests that BETi (AZD5153, BMS-986378) decreases the amount of active β-catenin in metastatic OS TT2 xenoline. Studies are in progress to evaluate mechanisms-of-action that link BET inhibition to modulation of the Wnt/β-Catenin pathway and to determine generality of pathway downregulation as a biomarker of therapeutic response to BETi in models of OS.

Translational/Clinical Research ***Post-Baccalaureate Fellow***

POSTER #63

ELUCIDATING THE ROLE OF IKBKB IN PIK3CA MUTATION-DRIVEN BREAST CANCERS

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PIK3CA is the second most mutated and/or amplified gene in breast cancer after p53. Mutations are frequently found in the kinase domain (H1047R) and helical domain (E542K and E545K). The PIK3CA-specific inhibitor Alpelisib is an FDA approved treatment for breast cancer. Toxicity and rapid development of resistance limit its clinical utility. Therefore, additional therapeutic targets for *PIK3CA*-mutated breast cancer need to be identified to overcome drug resistance. Our laboratory has focused on developing breast cancer models that harbor breast cancer-enriched mutant oncogenes using breast tissues of healthy women. In this context, the laboratory has shown that $PIK3CA^{H1047R}$+ SV40-T/t antigen transformed immortalized epithelial cells develop non-metastatic adenocarcinomas while $H\text{-}Ras^{G12V}$+SV40-T/t antigen transformed cells develop adenocarcinomas with lung metastasis. While $H\text{-}Ras^{G12V}$ mutants could transform breast epithelial cells without SV40-T/t antigens, $PIK3CA^{H1047R}$ alone was incapable. However, breast epithelial derived from *BRCA1/2* mutation carriers could easily be transformed by $PIK3CA^{H1047R}$ mutant. These results suggest that transformation by $PIK3CA^{H1047R}$ requires additional genomic aberrations like those caused upon SV40-T/t antigens overexpression or *BRCA1/2* mutations. Through extensive literature search and tumor genomic analyses, we are investigating three aberrantly expressed genes that may cooperate with mutant *PIK3CA* in transformation: *IKBKB*, *IKBKE* and *Notch1*. We observed ~20% of metastatic breast cancers harbor *PIK3CA* mutation and *IKBKB* or *IKBKE* aberrations (two endogenous activators of NF-kB). Given the role of NF-kB in metastasis and drug resistance, this project will test the hypothesis that *IKBKB* co-amplified with mutant $PIK3CA^{H1047R}$ is responsible for metastatic progression and resistance to Alpelisib. In this respect, we have observed increased IKBKB activity in $H\text{-}Ras^{G12V}$+SV40-T/t antigen but not in $PIK3CA^{H1047R}$+ SV40-T/t antigen tumor derived cells. To test the above hypothesis, we have generated breast epithelial cell lines that express $PIK3CA^{H1047R}$ +SV40-T/t, *IKBKB*+SV40T/t or $PIK3CA^{H1047R}$+*IKBKB*+SV40T/t antigens. Studies will evaluate the role of amplified *IKBKB* in metastasis of PIK3CA transformed cells as well as determine drug sensitivity *in vivo*. Experiments will be performed to determine therapeutic efficacy of concurrent NF-κB and PIK3CA inhibition. The laboratory has previously generated a NF-kB inhibitor called DMAPT with *in vivo* therapeutic activity, which will be tested with and without Alpelisib. The findings from the proposed study will reveal the role of IKBKB in breast cancer metastasis and potential therapeutic target for *PIK3CA* mutated breast cancers that are resistant to Alpelisib.

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

POSTER #64

DLD IS A POTENTIAL THERAPEUTIC TARGET FOR COVID-19 INFECTION IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS

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Since the discovery of copper induces cell death(cuprotosis) in 2022, it has been one of the biggest research hotspots. cuprotosis related genes (CRGs) has been demonstrated to be a potential therapeutic target for cancer, however, the molecular mechanism of CRGs in coronavirus disease 2019 (COVID-19) infected in DLBCL patients has not been reported yet. Therefore, our research objective is first to elucidate the mechanism and role of CRGs in COVID-19. Secondly, we conducted univariate and multivariate analysis and machine learning to screen for CRGs with common expression differences in COVID-19 and DLBCL. Finally, the functional role and immune mechanism of genes in DLBCL were confirmed through cell experiments and immune analysis. The research results show that CRGs play an important role in the occurrence and development of COVID-19. Univariate analysis and machine learning confirm that dihydrolipoamide dehydrogenase (DLD) is the common key gene of COVID-19 and DLBCL. Inhibiting the expression of DLD can significantly inhibit the cycle progression and promote cell apoptosis of DLBCL cells and can target positive regulation of Lysine-specific demethylase 1 (LSD1, also known as KDM1A) to inhibit the proliferation of DLBCL cells and promote cell apoptosis. The immune analysis results show that high-expression of DLD may reduce T cell-mediated anti-tumor immunity by regulating immune infiltration of CD8 + T cells and positively regulating immune checkpoints LAG3 and CD276. Reducing the expression of DLD can effectively enhance T cell-mediated anti-tumor immunity, thereby clearing cancer cells and preventing cancer growth. In conclusion, DLD may be a potential therapeutic target for COVID-19 infection in DLBCL patients. Our research provides a theoretical basis for improving the clinical treatment of COVID-19 infection in DLBCL.

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

POSTER #65

IMPROVING ANTI-MYELOMA EFFICACY OF T CELL ENGAGER BY SYNERGISTIC COMBINATION WITH PD-L1 INHIBITOR

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Improving anti-myeloma efficacy of T cell engager by synergistic combination with PD-L1 inhibitor

Background: T-cell-engaging immunotherapy holds promise for treating relapsed refractory multiple myeloma (MM), despite facing challenges from initial and acquired resistance partly attributed to the PD-1/PD-L1 signaling pathway, which can suppress T-cell activity. While PD-1 and PD-L1 neutralizing antibodies have shown limited efficacy in MM, they can enhance T cell-mediated killing of MM cells. To address these limitations, we have developed a novel PD-L1 binding peptide conjugated with a mitochondrial-targeted peptide (klaklak)2, offering superior specificity and tissue penetration compared to PDL1 antibodies. This construct, known as PD-L1pep2-k, not only inhibits PD-L1 but also triggers the intracellular release of the (klaklak)2 peptide upon internalization. This leads to mitochondrial depolarization and subsequent cell death. We hypothesize that combining PD-L1pep2-k with T-cell-engaging immunotherapy will enhance MM cell killing in myeloma.

Objectives: We evaluated PD-L1pep2-k both independently and in combination with teclistamab, an approved BCMA xCD3 bispecific antibody, using an in vitro culture and co-culture model mimicking cancer cell engraftment on bones. Our analysis included assessing tumor killing, T-cell activation, and mechanisms of cell death.

Results: In the cytotoxicity assay, PD-L1pep2-k showed an IC50 of 8.04 ± 0.61 μM. Using NCI-H929 MM cells cultured with human CD3 T cells, PD-L1pep2-k demonstrated increased cytotoxicity compared to cultures without human T cells, while PD-L1pep2-k had no impact on T cells alone, suggesting that PD-L1pep2-k counteracted PD-L1-mediated T-cell suppression. In co-cultures of human CD3+ T cells and MM cell lines, PD-L1pep2-k at a dose below IC50 (2 μM) in combination with teclistamab (1 nM) significantly enhanced cytotoxicity compared to individual agents. Additionally, this combined treatment promoted T cell activation, evidenced by the heightened presence of CD8+CD25+ cytotoxic T cells, compared to each agent alone. PD-L1pep2-k cell death mechanism might involve immunogenic cell death (ICD), shown by rapid calreticulin buildup on MM cell membranes after treatment, a key ICD feature. To assess the combined efficacy in a tumor microenvironment that may confer therapy resistance, we evaluated PD-L1pep2-k in MM cells co-cultured with mouse calvariae. The adhesion of MM cells to the bone caused a slight shift in the IC50 of PD-L1pep2-k, but it remained within the physiologically relevant range. Ongoing experiments aim to incorporate T cells into the MM: bone co-culture system to examine the combination of PD-L1pep2-k and T cell-engaging antibodies.

Conclusion: PD-L1pep2-k demonstrates efficacy against MM cells by directly inducing cytotoxicity and activating T cells. When combined with teclistamab, it enhances tumor elimination and has the potential to disrupt signals in the bone marrow microenvironment that support MM cell survival. Moreover, PD-L1pep2-k may trigger immunogenic cell death, enhancing immune responses against MM cells. Ongoing studies will further evaluate PD-L1pep2-k using primary tumor cells and animal models, aiming for future clinical trials in combination with other T-cell immunotherapies.

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

POSTER #66

SMALL MOLECULE RPA INHIBITORS ABROGATE ATR KINASE SIGNALING

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The ATR kinase is activated upon elevated levels of single-stranded DNA (ssDNA), or replication stress, to direct the response that alleviates the ssDNA burden and activates the G2/M checkpoint signaling pathway, thereby ensuring DNA replication is complete and genomic material is faithfully passed to daughter cells. Thus, this pathway has been therapeutically targeted as oncogenic cells possess intrinsically elevated levels of replication stress. The ssDNA lesions that are sensed by ATR are bound to the ssDNA-binding protein RPA, which acts as a platform that interacts with ATRIP for recruitment and subsequent activation of ATR. We have developed RPA inhibitors (RPAi) and RPA70 OB-F inhibitors (RPA-Fi) that block RPA-ssDNA and protein-protein interactions, respectively, both of which are required for ATR activation. Here, we biochemically reconstitute the RPA-, ssDNA-, and TopBP1-dependent ATR kinase pathway. We demonstrate that both RPAi and RPA-Fi disrupt ATR-dependent phosphorylation events. Lastly, we examine the impact of RPA phosphorylation and acetylation on ATR kinase activation and sensitivity to RPAi. We find that phosphorylation of RPA and TopBP1 stimulate ATR activity, while RPA acetylation has no effect. Collectively, this works describes a mechanism of action of RPAi/RPA-Fi whereby RPA inhibition induces replication catastrophe by blocking ATR kinase activity.

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

POSTER #67

THE LOSS OF BOTH ISOFORMS OF RAP1A AND RAP1B IN OSTEOBLASTS AFFECTS THE NON-AUTONOMOUS MATURATION OF B-LYMPHOID AND HEMATOPOIETIC STEM CELL FUNCTIONS.

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Rap1 (Ras-proximity-1) is a small GTPase protein within the Ras superfamily, pivotal in cellular functions like adhesion, proliferation, differentiation, and migration. It can switch between an inactive GDP-bound state and an active GTP-bound state, which is facilitated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). Rap1 exists in two isoforms, Rap1a and Rap1b, which share structural similarities with distinct functions. While Rap1 has been implicated in regulating several hematologic disorders, its role in the development and function of hematopoietic stem and progenitors (HSC/Ps) has not been investigated. Despite the extensive knowledge about Rap1 functions in different cell types, its combined deficiency of both isoforms of Rap1 in the context of cell-autonomous and non-autonomous hematopoietic function is known very little. Given spatially distinct endosteal ‘osteoblastic’ niche regulating stem cell function and B lymphoid development and Rap1's ubiquitous expression in various cell types, as well as its role in hematopoiesis, we hypothesized that Rap1 within the osteoblastic niche plays a crucial role in influencing growth and development of stem cells. To investigate the role of Rap1 in the osteoblastic niche, we have conditionally deleted both the isoforms of Rap1 specifically in osteoblasts individually or in combination (double knockout; DKO) by crossing with Col.Cre2.3.

Our phenotypic characterization of ccRap1DKO mice unveiled an elevated frequency of leukocytes, coupled with a notable reduction in lymphocytes in peripheral blood. Flow cytometry analysis demonstrated an expansion of Lin-Sca1+c-Kit+ (LSK) cells and myeloid cells, as well as a hindered B cell differentiation in Rap1DKO mice compared to WT mice. Competitive and non-competitive transplantation assays reveal reduced stem cell engraftment in Rap1DKO mice, along with decreased lymphocytes and increased neutrophils, mirroring the primary DKO mouse phenotype, highlighting Rap1's involvement. CODEX multiplex imaging confirms B cell maturation impairment in the spleen of Rap1DKO mice. Molecular analyses using mRNA sequencing of osteoblast cells unveil elevated TNF signaling and NF-kB expression in Rap1DKO mice. Additionally, mRNA sequencing data from LSK cells of mice with osteoblast specific Rap1DKO show increased expression of pro-survival genes. Our study demonstrates that deleting both isoforms of Rap1 in osteoblasts effects stem cell function, and hinders early Pre-B stage B cell differentiation, offering insights into the non-autonomous functions of Rap1.

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

POSTER #68

IMPACT OF OPTIMIZED KU-DNA BINDING INHIBITORS ON THE CELLULAR AND IN VIVO DNA DAMAGE RESPONSE TO DSB GENERATING THERAPIES.

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DNA-PK, the DNA-dependent protein kinase, is a validated target for cancer therapeutics that drives the DNA damage response (DDR) and plays a critical role in the non-homologous end joining (NHEJ) repair of ionizing radiation (IR) induced DNA double-strand breaks (DSB). The Ku 70/80 heterodimer initiates the process as the DNA-binding component of DNA-PK. Modulating the DSB repair pathways through blocking the DNA-PK catalytic activity in combination with DSB-inducing agents has been widely used as a therapeutic strategy to drive the clinical efficacy of radiation therapy and certain DNA damaging chemotherapeutic drugs used to treat cancer. We have reported a unique approach to DNA-PK inhibition by developing Ku-DNA binding inhibitors (Ku-DBi's), small molecule inhibitors that target the interaction between Ku70/80 and DNA. Ku-DBi's demonstrated a direct interaction with Ku70/80, a potent inhibition of Ku-DNA binding and DNA-PK catalytic activity, and *in vitro* and cellular NHEJ inhibitory activity. This results in the potentiation of non-small cell lung cancer (NSCLC) cellular sensitivity to DSB-generating therapies, as a function of inhibiting DNA-PKcs autophosphorylation and dysregulation of signaling to the DDR. In this study, we have expanded our structure- activity relationship analyses to focus on optimizing selectivity, cellular uptake, and Ku inhibitory activity. Our recent chemical optimization has identified independent pharmacophores that displayed improved cellular uptake while retaining potent Ku inhibitory activity and enhanced cellular inhibition on DNA-DSB repair as a function of new pharmacophore additions. This optimization enabled Ku-DBi's assessment *in vivo* analyses of Ku-DBi - IR combination therapy in human xenograft models of non-small cell lung cancer. These data represent a significant advance in the development of Ku-DNA binding inhibitors towards interrogating NHEJ and DDR signaling *in vivo* and their therapeutic intervention for cancer treatment.

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

POSTER #69

INVESTIGATING THE INTERACTION BETWEEN ETS FAMILY MEMBERS AND MUTANT P53

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Cancer cases are on the rise globally requiring a deeper understanding of the disease and identification of novel therapeutic targets. Mutations in genes often result in tumor formation. TP53 encodes p53, a tumor suppressive transcription factor, and is mutated in nearly 50% of all cancer cases. Once mutated, p53 loses its tumor suppressive function while simultaneously gaining oncogenic function. p53 mutations disrupt direct p53 binding to DNA and promotes p53 binding to regulatory regions of oncogenes via protein-protein interactions with other transcription factors. One of these interacting partners is ETS2, a member of the ETS transcription factor family. ETS binding sites are present in 50% of all mutant p53 occupied regulatory elements. Other ETS family members have also been linked to mutant p53 but these interactions have either been deemed weak (ETS1) or have yet to be identified as direct (ERG). It is my aim to determine the scope of interaction between the ETS family and mutant p53 and to elucidate whether this interaction is required for the observed oncogenic phenotypes of ETS proteins and mutant p53.

To determine which ETS proteins interact with mutant p53 purified ETS proteins were used for affinity pull-down of purified mutant p53. The entire ETS family interacted with mutant p53 to some degree, and several ETS proteins had stronger interactions than ETS2. Truncation studies were used to determine interaction domains. One interacting region was the conserved DNA binding domain, which may explain interaction across the entire family. ERG and ETS2, both strong mutant p53 interactors, had two interaction interfaces. I hypothesize that the second interaction interface defines strong interactors. To determine if interacting ETS are responsible for targeting mutant p53 to the genome, chromatin immunoprecipitation sequencing measured mutant p53 binding in the presence or absence of different ETS factors. These data indicate that each of the conditions resulted in different p53 binding patterns and that there is a requirement for ETS in mutant p53 binding. Lastly, I wanted to determine if there was a correlation between p53 mutation and expression of strong mutant p53 interactors in certain cancer types. Analysis of TCGA samples showed that in ovarian cancers with p53 mutations, ETS factors that are strong mutant p53 interactors were upregulated in a mutually exclusive pattern, while weak interactors tended to be downregulated/unchanged. Similar trends were observed among some other cancer types.

My studies have shown that several ETS proteins interact with mutant p53 in vitro, recruit p53 to the genome, and that this interaction pattern correlates with expression in mutant p53 driven cancers. Additionally, studies are ongoing to determine phenotypes related to this interaction. Ultimately, if ETS/mutant p53 interactions are deemed important for oncogenesis, these will be attractive targets for drug development.

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

POSTER #70

TARGETING OLIGOSACCHARYLTRANSFERASE COMPLEX SENSITIZES MM CELLS TO BORTEZOMIB TREATMENT

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Multiple myeloma (MM) is characterized by the clonal expansion of mutated plasma cells within the bone marrow. Despite advancements in therapy, relapsed MM remains incurable, underscoring the urgent need for identifying novel therapeutic targets. The oligosaccharyltransferase (OST) complex, responsible for glycan transfer to nascently translated proteins' asparagine residues, represents a pivotal step in N-glycosylation. In this study, we identified the OST complex as a novel vulnerability in MM cells. We found that MM cells highly express the OST complex, which is associated with relapsed MM and poor prognosis. Moreover, patients with high expression levels of the OST complex were strongly associated with known high-risk factors of MM (biallelic deletion of TP53, amplification of 1q, t(4;14), and t(14;16)). Disruption of the OST complex significantly impedes MM cell growth by inducing cell cycle arrest and promoting apoptosis. Notably, combining OST complex inhibition with bortezomib synergistically eradicates MM cells (cell lines and patient-derived cells), concurrently suppressing genes associated with bortezomib-resistant phenotypes. Notably, we demonstrated that this combination suppressed MM tumor growth in vivo using the subcutaneous xenograft model.

Mechanistically, OST complex disruption in MM cells leads to the downregulation of pathological transcriptomic signatures, including NF-kB signaling, the mTORC1 pathway, glycolysis, MYC targets, and cell cycle progression. Furthermore, it instigates TRAIL-mediated apoptosis and inflammatory pathways. Collectively, our findings underscore the OST complex as a novel therapeutic target for MM. Combining OST complex inhibition with bortezomib presents a promising avenue for effectively treating relapsed MM patients, offering potential therapeutic benefits beyond current treatment modalities. We are currently investigating functions of each subunit of the OST complex in MM pathology and identifying direct substrates of the OST complex that might be vulnerabilities in MM cells.

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

POSTER #71

DEVELOPMENT OF ORTHOTOPIC MOUSE MODEL FOR COLORECTAL CANCER USING NON-SURGICAL APPROACH

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Efforts to replicate the physiologically relevant tumor microenvironment represent a significant hurdle in modeling colorectal cancer (CRC) for both basic research and clinical translation. While the cecal-wall injection technique is commonly utilized to establish CRC animal models, its reliance on intricate surgery and unnatural tumor placement presents substantial challenges for therapeutic development. Other models like subcutaneous injection model have been widely used in the literature, although, suffer from the drawback of representing an ectopic environment and lacking metastasis. To overcome these limitations, we are reporting a non-surgical approach for developing orthotopic CRC mouse models, aiming to better emulate disease progression and enhance translational studies, including drug screening and validation. In this procedure, anesthesia is induced in overnight fasted study immunocompetent or immunodeficient mice while placed on a thermostat-controlled heating blanket. A specialized catheter is inserted and distended into the mouse colon to gently block the colon and ensure the localization for tumor development. Hereafter, the colonic epithelium is treated with proteases and mechanical abrasion to cause mild disruption. Following the local gut inflammation, the targeted colon region is incubated with tumor cells, leading to the adhesion of tumor cells to the colonic epithelium. Based on the animal background and the cell line used, the tumor developed within 3-4 weeks with a success rate ranging from 70-90%. The stages of tumor development at different time points were successfully confirmed using hematoxylin and eosin stain. Taken together, this non-surgical approach encompasses technical barriers associated with surgery and offers advantages such as ease of implementation, cost-effectiveness, pain-free execution with minimal procedure-related mortality, and adaptability to various strains. Further, tumor microenvironment analysis revealed a significant increase in immune cell infiltration, particularly CD4+ T-cells and tumor-associated macrophages in non-surgical procedure compared to the cecal-wall injection method. Additionally, heightened PD-1 expression on CD8+ T-cells in the cecal-wall injection compared to non-surgical model underscores the potential impact of tumor site on the efficacy of immune checkpoint inhibitors and therapeutic outcomes. Moreover, the immune profiling data affirmed the resemblance of non-surgical model with human colorectal cancer. In addition, this procedure can be implicated in tumor development in diverse mouse strains, local/orthotopic delivery of adeno-Cre in transgenic mice for CRC suitable for targeted therapies, and delivering tumor organoids and patient-derived xenografts in immunodeficient mice. In conclusion, our non-surgical methodology presents a promising alternative to traditional surgical orthotopic CRC mouse models, facilitating more efficient drug development and translational research endeavors.

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

POSTER #72

TARGETING NQO1 WITH AN NQO1 BIOACTIVABLE DRUG, IB-DNQ: A BREAKTHROUGH IN PRECISION CANCER THERAPY FOR ENHANCED EFFICACY AND REDUCED TOXICITY

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Cancer remains a significant global health challenge, with tumor progression being a leading cause of mortality. The quest for effective anticancer therapies is paramount, as traditional treatments often induce severe toxic side effects. This study introduces a novel therapeutic approach targeting NQO1, a promising biomarker distinctly overexpressed in various cancer types, including breast, lung, and colon cancers, yet minimally present in healthy tissues. We investigated the action of an innovative NQO1 bioactivable drug, IB-DNQ, demonstrating remarkable efficacy and tumor specificity upon application to cancer cells. IB-DNQ exploits the elevated NQO1 expression in tumor cells to induce selective cytotoxicity through the generation of reactive oxygen species (ROS), thereby initiating oxidative stress and subsequent immune-mediated tumor suppression. Our research delineates the mechanistic pathway of IB-DNQ, revealing its capability to activate tumor-intrinsic innate sensing pathways and Th2-mediated adaptive immune responses, based on the selective catalysis by NQO1 within the tumor microenvironment (TME). The study highlights the therapeutic potential of targeting NQO1 and positions IB-DNQ as a significant breakthrough in cancer precision medicine, offering a strategy that specifically attacks NQO1-expressing tumors with minimal impact on normal tissue.

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

POSTER #73

ESTABLISHING A HIGH-THROUGHPUT METHOD OF CELLULAR SENESCENCE QUANTIFICATION IN OVARIAN GRANULOSA CELLS

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Cellular senescence is a unique type of growth arrest characterized by a lack of proliferation and apoptosis. It can be attributed to DNA damage, including cellular stresses from reactive oxygen species, chemotherapy, physiologic aging, or oncogene expression, particularly oncogenic KRAS-mediated cellular senescence. We believe cellular senescence is the primary underlying mechanism by which our novel mouse model develops spontaneous ovarian endometriomas. Ovarian endometriomas are deeply invasive endometriosis lesions that contain endometrial-like tissue that develops into blood-filled cystic lesions of the ovary. While endometriomas can result in significant pelvic pain and infertility, ovarian endometriomas portend a 10-fold increased risk of ovarian cancer. Transcriptomic profiling of mouse ovarian endometriomas showed an enrichment of cellular senescence-associated genes. Further, immunohistochemistry showed intense staining with CDKN2A in ovarian granulosa cells. We hypothesize that granulosa cells are undergoing cellular senescence before endometriosis formation. This study aims to develop a high-throughput method of quantifying cellular senescence from granulosa cells. In our mouse model of ovarian endometriomas, the lesion origin is likely granulosa cells. Because mouse granulosa cells only survive a short time in culture, we began our assay optimization with an invasive ovarian granulosa cell line, KGN.

To understand how endometrial pathogenesis may impact the development of ovarian cancer, a human granulosa-like tumor cell line (KGN) serves as a model of ovarian granulosa cells. Initial assessment by β-galactosidase stainingshowed that dosage-dependent treatment with hydrogen peroxide (H_2O_2) triggers KGN cell senescence via oxidative stress. Cells treated with 5uM H_2O_2 for a maximum of two hours exhibit at least a 10-fold increase in percent positivity of senescent cells in comparison to untreated cells ($n=3$, $p<0.05$).To further optimize KGN cell senescence and the validity of the β-galactosidase activity assay used to monitor senescence, future studies are focused on a comparative analysis between oxidative stress by H_2O_2 and camptothecin, a known topoisomerase I inhibitor. With this work, we will establish a model for ovarian granulosa cell senescence, which will warrant further assessment of oncogenic-driven senescence in both our human and genetically modified mouse models of endometriosis and endometriosis-associated ovarian cancers.

Basic Science ***Research Technician***

POSTER #74

NEUROMUSCULAR DYSFUNCTION PRECEDES MUSCLE ATROPHY IN COLORECTAL CANCER

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Background: Cancer patients frequently develop skeletal muscle wasting and weakness, hallmarks of cachexia, a wasting disease which worsens quality of life and is directly responsible for up to 30% of all cancer-related deaths. While advancements in detection and treatment have increased the population of cancer survivors, skeletal muscle dysfunction can persist for years following cancer remission. We previously demonstrated that late-stage cachexia is associated with impaired skeletal muscle innervation, linking loss of motor unit (MU) connectivity to cancer-induced wasting and weakness. In the present study, we investigated the onset of neuromuscular dysfunction in a preclinical model of colorectal cancer cachexia. **Methods:** CD2F1 male mice (8-week-old) were injected subcutaneously (intrascapular) with murine C26 colorectal cancer cells ($1.0x10^6$) or saline and randomized into one the following timepoint groups: day 6, day 8, or day 10 (n=8-10). Animals were assessed for indices of MU connectivity and muscle contractility at each timepoint. Following functional assessment, skeletal muscles were harvested, weighed, and processed for molecular analyses. **Results:** 6 days post tumor injection, C26 hosts displayed reductions in neuromuscular junction (NMJ) transmission and motor unit connectivity, while absolute muscle torque and muscle mass were preserved. Specific muscle torque was reduced in C26 hosts at day 8, while reductions in muscle mass or cross-sectional area did not occur until day 10. Molecular analysis revealed alterations of NMJ components as early as day 6 in C26 hosts, further suggesting that neuromuscular dysfunction precedes muscle atrophy. **Conclusions:** Altogether our data demonstrate that cancer-induced neuromuscular dysfunction precedes cancer-induced muscle atrophy, identifying impaired innervation as an early prognosticator of cachexia progression. Our work supports strategies to counteract impaired neuromuscular function in the treatment of cancer cachexia, in hopes of sustaining quality of life in cancer patients and the growing population of cancer survivors.

Basic Science ***Research Technician***

POSTER #75

CD73 INHIBITOR IN COMBINATION WITH HIF-1ALPHA INHIBITOR PREVENTS HYPOXIA-MEDIATED ESOPHAGEAL CANCER INVASION AND ANGIOGENESIS

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Background: Esophageal adenocarcinoma (EAC) is one of the most aggressive human cancers with increasing incidence in the United States. The prognosis for this cancer remains poor, and most patients eventually die even with modern combination therapies. We hypothesize that the tumor's hypoxic microenvironment-induced overexpression of hypoxia-inducible factor-1alpha (HIF-1α) and CD73, an immunosuppressive enzyme, plays a critical role in aggressive EAC growth, invasion, and angiogenesis thereby causing therapeutic resistance. In this study, we have investigated the combination effect of a CD73 inhibitor (SBP12379) and a HIF-1 inhibitor (acriflavine) on hypoxia-induced EAC cell growth, invasion, and angiogenesis.

Methods: First, we performed a western blot assay to evaluate the hypoxia-induced expression of HIF-1a and CD73 in EAC cell lines (Flo-1 and OE19). Second, we performed a Matrigel-coated Boyden Chamber assay to evaluate hypoxia-induced EAC cell invasion. Cell migration was evaluated by scratch-wound healing assay. Antiproliferative activities of acriflavine and PSB12379 on EAC cells were measured by Cell Titer Glo luminescence assay (Promega). ELISAs were performed to measure VEGF and CD73 in condition media. The antiangiogenic activity of the inhibitors was evaluated by HUVEC tube formation assay.

Results: Western blot data reveal that acriflavine prevented hypoxia-induced HIF-1a expression in EAC cells, as expected. CD73 and VEGF production by hypoxic EAC cells were prevented by both acriflavine and PSB12379. Acriflavine and PSB12379 dose-dependently inhibited hypoxia-induced EAC cell invasion, and this inhibition was significantly enhanced when cells were treated with both drugs. HUVEC tube formation is the direct evaluation of *in vitro* cancer angiogenesis. The angiogenesis assay showed a reduction in vascular formations with increasing doses of PSB12379 and acriflavine. However, PSB12379 treatment in combination with acriflavine completely prevented vascular formation.

Conclusions: In conclusion, our data suggest that simultaneous inhibition of HIF-1a and CD73 by acriflavine and PSB12379, respectively, could be an important therapeutic approach for esophageal adenocarcinoma and perhaps other solid cancers.

Basic Science ***Research Technician***

POSTER #76

JP11646-MEDIATED PIM2 INHIBITION HAS POTENT ANTITUMOR EFFECTS IN SMALL CELL LUNG CANCER AND LARGE CELL NEUROENDOCRINE CARCINOMA OF THE LUNG

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Introduction:

Small cell lung cancer (SCLC) is an aggressive neuroendocrine (NE) lung carcinoma characterized by acquired chemoresistance at relapse. SCLC tumor plasticity and chemoresistance is driven, at least in part, by the downstream effects of MYC amplification or overexpression, namely activation of Notch and subsequent induction of NE dedifferentiation. Despite increased molecular characterization of SCLC, few effective options at relapse are available, highlighting the urgency for novel therapeutics, namely those that target MYC-dependent pathways. C-MYC is stabilized by the proviral integration site for Moloney murine leukemia virus 2 (PIM2) kinase through phosphorylation. A novel pan-PIM non-ATP competitive inhibitor JP11646 has shown promising antitumor activity in various cancers; however, JP11646 has not yet been studied in SCLC. Here, we report the antitumor efficacy of JP11646 in MYC-amplified SCLC and large cell neuroendocrine carcinomas (LCNEC).

Methods:

Cell culture/reagents: Human SCLC lines (H209, SHP-77, H1417, H187, H2171, H69, H524) and LCNEC line H1155 were obtained from ATCC, cultured in RPMI-1640 with 10% fetal bovine serum, and incubated at 37°C in 5% CO_2. JP11646 (10 mM, Jasco Pharmaceuticals) was gifted by Dr. Kelvin Lee.

Drug sensitivity assay in vitro: A total of 1,000 cells/well were seeded in a 384-well plate and incubated overnight at 37°C. Serial dilutions of JP11646 (1 nM - 10 μM) were added in triplicate with 2-3 biological replicates and incubated for 72 h at 37°C. Viability was measured with Promega CellTiter-Glo™ Luminescent Assay Kit.

Effects of JP11646: JP11646 (or H2O) was added at 500 nM to 2 x 10^6 cells for 24 hours and harvested for WB.

Western blot analysis: Cell lysates were generated per standard protocol. Western blot performed with the BioRad Trans-Turbo system per standard protocol. Membranes were blocked with EveryBlot Blocking Buffer (EBB) for 5 minutes, primary 1:1000 in EBB for 30 minutes, washed, secondary 1:5000 in EBB for 30 minutes, washed, and exposed with ECL reagent. Antibodies utilized: PIM2 (D1D2, CST), C-MYC (D84C12, CST), N-MYC (D4B2Y, CST), and vinculin (E1E9V, CST).

Results:

PIM2 protein is expressed in SCLC and LCNEC. Across the panel of SCLC and LCNEC, the mean IC_{50} for JP11646 was 87 nM. MYC paralog-amplified cell lines' IC_{50} ranged from 7 nM to 117 nM, with C-MYC amplified H524 harboring exquisite sensitivity. With JP11646 treatment, we find that PIM2 protein expression decreases. Furthermore, MYC paralog (C-MYC, N-MYC) protein expression decreases within 24 hours of JP11646 treatment across the panel.

Conclusion:

PIM2 is expressed in neuroendocrine carcinomas and serves as a potential therapeutic target for relapsed disease. Treatment with a PIM2 inhibitor effectively inhibits cell viability in SCLC and LCNEC. JP11646 leads to reductions in MYC paralogs. Validation with xenograft models and exploration of downstream effectors of MYC with JP11646 are planned.

Basic Science ***Research Technician***

POSTER #77

LEUCINE SUSTAINS CANCER CELL SURVIVAL AND PROLIFERATION IN THE ABSENCE OF GLUCOSE

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Glucose availability regulates the progression of cancer. Malignant cells preferentially consume glucose to accelerate tumor growth. As a result, the tumor microenvironment is usually glucose deficient. Such dependence on glucose leads to tumor cells seeking alternative sources of energy. For example, cancer cells can utilize proteins and certain amino acids to fuel survival pathways. Amino acids such as methionine, glycine, and cysteine are used by malignant cells. Researchers have extensively studied the roles of these amino acids but the biological function of the amino acid leucine in cancer is yet to be determined. 3-Hydroxy-3-methylglutaryl-CoA lyase degrades leucine, an essential branched-chain amino acid, into acetoacetate and acetyl CoA which are vital energy substrates. Herein, we aim to investigate whether leucine supplementation can provide a survival advantage for cancer cells in the absence of glucose. Thus, we examined whether adding leucine to cells growing in a glucose-deficient medium affects proliferation. Because of the interdependence of glutamine and glucose metabolism, we also performed our experiments in the absence of glutamine. Our findings suggest leucine increases survival and proliferation of prostate cancer cells without glucose and glutamine. This suggests that leucine is essential for survival of prostate cancer cells under nutrients-restricted conditions. Future studies will focus on determining the role of 3-Hydroxy-3-methylglutaryl-CoA lyase in mediating the ability of leucine to regulate cancer cells proliferative capacity. Overall, cancer cells use leucine to support their survival and growth, creating new possibilities for nutrition and drug therapies for cancer patients.

Basic Science ***Research Technician***

POSTER #78

GLOBAL PROTEOMICS AND INTEGRATIVE THERMAL PROTEOME PROFILING OF HEPATIC STELLATE CELLS IN LIVER FIBROGENESIS

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1/400 adults have cirrhosis, a disease where chronic liver damage leads to fibrotic scarring, impaired liver function, and increased risk of cancer. A major contributor to cirrhosis is dysregulated fibrogenesis driven by activation of Hepatic Stellate Cells (HSCs). Activated HSCs induce fibrogenesis by secreting extracellular matrix proteins to repair the damaged liver and then are either cleared or become quiescent. Chronic liver injury leads to fibrosis through dysregulated fibrogenesis without clearance of activated HSCs. A connection has been established between the unfolded protein response (UPR) and fibrogenesis in HSC. Chronic ER stress and UPR signaling may alter protein homeostasis in HSCs, preventing the apoptosis of activated HSCs. Integrative thermal proteome profiling (PISA) and other proteomics approaches may provide additional insights.

Primary HSCs were grown to similar densities and treated with either TGFβ (8Hr, 24Hr) to induce HSC activation or Tunicamycin (8Hr) to induce UPR. Vehicle treatments were created for each time point in 1mg/ml BSA 4mM HCL. Treatment effectiveness was initially confirmed by Western Blotting. Samples were prepared for global proteomics and PISA. These datasets will be analyzed through mass spectrometry-based proteomics on an Orbitrap Eclipse and antibody-based Olink proteomics. For quantitation, temporal treatment studies will be directly paired with their corresponding vehicle sample. MS samples will be labeled with tandem mass tags and be analyzed for quantitation and statistics using Proteome Discoverer (Thermo).

The UPR can be signaled by three canonical ER stressors: ATF6α, IRE1α, and PERK, which signal through a distinct mechanism to regulate proteostasis. Western blot analysis showed nearly a 3-fold increase in the IRE1α-downstream protein XBP1s in response to tunicamycin, and TGF-β significantly increased the expression of phosphorylated IRE1. Furthermore, PERK has also been shown to initiate fibrogenesis by utilizing ATF4 in HSC-T6 cells. While the role of ATF4 in fibrogenesis and HSC activation has yet to be fully researched, a greater than 2-fold increase was observedin ATF4 when cells were treated with tunicamycin. The treatments described have been linked to statistically significant changes in relevant proteins. Collagen production increased 3-fold in TGF-β treated samples, and a similar response was observedfollowing treatment with tunicamycin at 8 hrs. Other markers such as binding immunoglobulin protein (BiP), a chaperone protein in the endoplasmic reticulum, had nearly a3-fold increase in expression following treatment with tunicamycin based on Western blot analysis. Fibronectin, a protein involved in tissue repair, undergoes a 3-fold increase in TGF-β samples. Taken together, the analyses revealed both tunicamycin and TGF-β significantly upregulated collagen, fibronectin, and BiPexpression, indicatingan activated stress response. Further work using mass-spectrometry and Olinkwill be presented and are expected to offer insights into the role of the UPR and the integrated stress response in liver fibrogenesis via HSCs.

Basic Science ***Research Technician***

POSTER #79

REGULATION OF OVARIAN CANCER METASTASIS BY THE MICROENVIRONMENT-INDUCED ETS1-EHD1 AXIS

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Ovarian cancer (OC) is the gynecologic malignancy with the highest mortality rate due to widespread metastases at diagnosis. Developing successful treatment approaches requires a greater understanding of the regulation of metastatic colonization, a critical, rate limiting stage in metastasis. We have studied the regulation of OC metastatic colonization by using an in vitro organotypic culture model of the omentum – a common site of OC metastasis. We combine this approach with end point analysis of primary tumors and matched metastases from ovarian cancer patients to determine key transcription factors regulating early and advanced metastases. We show that reciprocal interactions between cancer cells and the metastatic microenvironment induce ETS1 expression in ovarian cancer. This induction is mediated by the activation of p44/42 MAP kinase signaling upon interaction with mesothelial cells at the metastatic site. Through secretome analysis, conditioned media experiments, and heterotypic co-culture models, we identified basic fibroblast growth factor (bFGF) and insulin-like growth factor binding protein 6 (IGFBP6) as key factors secreted by mesothelial cells, driving ETS1 induction in a paracrine manner. Treatment with recombinant human bFGF and IGFBP6 could mimic the effects of mesothelial cells on ETS1 expression in OC cells. Inhibiting this regulatory axis prevented ETS1 induction in OC cells. Analysis of single-cell RNA-seq data from high-grade serous ovarian cancer patient metastases confirmed the clinical relevance of our findings. Further studies revealed that ETS1, in turn, regulates the expression of its transcriptional target EHD1, which plays a crucial role in promoting metastatic colonization. We examined how the transcription factor ETS1 and its downstream effector EHD1 facilitate the colonization of ovarian cancer metastases through functional assays including migration, proliferation, and colony formation assays. In addition, we demonstrated that EHD1 plays an important role in recycling cell surface receptors of the OC cells, critical for maintaining paracrine and juxtacrine signaling with the microenvironment. Additionally, EHD1 also translocated Src from perinuclear region to the cell membrane, enabling activation of FAK. We confirmed this function with the help of EHD1 and ETS1 knock down experiments. Additionally, we observed a decrease in FGFR1 recycling when EHD1 is knocked down, confirming its role in maintaining the OC crosstalk with the microenvironment. In summary, our study reveals a previously poorly understood aspect of ovarian cancer metastasis regulation, unveiling the pivotal roles of ETS1 in driving metastatic colonization through its transcriptional target EHD1. These findings offer promising targets for therapeutic intervention aimed at mitigating metastatic spread and improving patient outcomes.

Basic Science ***Research Technician***

POSTER #80

DEVELOPMENT OF AN HPV+ CERVICAL CANCER XENOGRAFT MODEL

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Abstract Title:

Development of anHPV+ cervical cancer xenograft model

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To test the in vivo efficacy of novel HPV16 inhibitors, we soughtto develop a xenograft mouse model.Several cell doses (3-10x106 cells/injection) of established HPV16 positive SiHa and CaSki cervical squamous cell carcinoma cell lines with or without Matrigelwere tested in nude mice purchased from Jackson laboratory.Even at maximumcell numbers, minimal tumor growth was observedup until60 days. Next, we tested other immune compromised mouse models (NSG, and NSGS) as well as nude mice purchased from Enivgo laboratories(now Inotiv). While we observedtumor growth in all three mousestrainswith 10x106 SiHa cells, growth initiation took long (Day 50: 354±112 mm3; n=5). Previous research showedthat estrogen administrationincreases theincidence of cervical dysplasiain HPV16E6/E7 transgenic mice. Based upon those findings, we speculated that estrogen supplementation would enhance tumor growth. For this experiment, we used the Envigo nude mouse due to their simple breeding scheme. Mice were treated with estrogen and progesterone 7 days prior to cell implantation and continued for another 17 days. We observed significantly enhanced tumor growth in the estrogen treated group. Interestingly, additional treatment (>24 days) did not correlate with increasedtumor growth. We then established a new cell clone from anestrogen treated SiHa tumor, which we reimplanted into nude mice. This cell clone had far greater tumorigenicity than the parental SiHa with 5x106 cells resulting in tumors >1000 mm3 by 20-days post-implantation.Interestingly, treatment with estrogen did not further enhance tumor growth of this cell clone.We therefore tested the hypothesis that estrogenmodulates the host microenvironment rather than the HPV+ cervical cancer cells by treating nude mice 7 days prior to tumor implantationand 4 days post-implantationwith tamoxifen (25 mg/kg), an estrogen inhibitor. This limitedtreatmentduration was sufficient togreatly slow tumor growth (Day 40: Untreated vs. Tamoxifen: 1457±162 vs. 260±65 mm3). These data support anadverse role for continuousestrogen exposure on the tumor microenvironment, which may have implication for women on contraceptives. Further studies are needed to investigatethe co-incidence of cervical cancer and contraceptives.Overall, we discovered a highly tumorigenic subpopulation of SiHa cells, which greatly improvesthe feasibility and usability of this xenograft model to test novel HPV16 antivirals.

Basic Science ***Research Technician***

POSTER #81

ATAXIA TELANGIECTASIA AND RAD3-RELATED PROTEIN (ATR) IS AN EXPLOITABLE VULNERABILITY FOR SELECTIVE DEATH OF NON-SMALL CELL LUNG CANCER WITH ISOBUTYL-DEOXYNYBOQUINONE (IB-DNQ)

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Lung cancer is the leading cause of cancer-related death in the United States and Indiana, accounting for 1 in 5 fatalities. In 2024, the American Cancer Society predicted that there will be around 234,580 new cancer cases and 125,070 cancer deaths projected to occur in the United States. Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer and is lethal as it is harder to identify in earlier stages. While there are many treatment options available for lung cancer patients, it is important to develop tumor-selective treatments based on predictive biomarkers for precision medicine. Most cancer treatments are non-specific that kill off normal and cancer cells, especially when administered at high doses, causing debilitating side effects and drug resistance. An approach that we have explored to reduce toxicity towards normal cells is using NQO1-bioactivatable agents. NQO1 (NAD(P)H:Quinone Oxidoreductase 1) is an enzyme that is aberrantly over-expressed in lung cancer, which could serve as a potential tumor-selective biomarker for therapeutic response. Isobutyl-deoxynyboquinone (IB-DNQ) is an NQO1-bioactivatable drug that is "bioactivated" by NQO1 to form an unstable hydroquinone intermediate that immediately reverts to the parental quinone molecule in a futile cycle. We showed that this futile cycle causes hydrogen peroxide (H_2O_2) to form, causing oxidative DNA damage and single-stranded DNA breaks (SSBs) that lead to replication stress. Ataxia telangiectasia and Rad3-related protein (ATR) is a protein kinase that is involved in sensing replication-induced DNA damage and activating the DNA damage checkpoint in response to persistent SSBs, which are common intermediates formed during DNA damage response and repair. We hypothesize that ATR provides a critical mechanism of resistance and survival in NQO1-expressing NSCLC treated with IB-DNQ, which is an exploitable vulnerability to potentiate the tumor-selective lethal effect of low-dose IB-DNQ using ATR inhibitors. Using patient samples, we showed that NQO1 levels are higher and catalase levels are lower in tumors compared to normal tissue. We then used long term-survival 2D assays to show IB-DNQ kills NQO1-expressing NSCLC cell lines and spares normal lung that does not express NQO1. We showed that varying doses of IB-DNQ causes dose-dependent ROS-H_2O_2 formation. Using western blot, we showed that IB-DNQ treatment activates ATR and Bay1895344 (Elimusertib) inhibits its activation when combined with IB-DNQ. Using Flow cytometry, we treated cells individually and in combination to determine the number of TUNEL positive cells that have gone through apoptosis. Using 3D sp3heroid co-culture, we showed how our combination treatment strategy at low concentrations induced more tumor-selective death compared to individual treatments alone. Overall, our results show an exploitable vulnerability that is tumor-selective at low-dose which should help improve the lives of patients with NSCLC.
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Basic Science ***Research Technician***

POSTER #82

CHEMICAL INHIBITION OF RPA SENSITIZES BRCA1 DEFICIENT CANCERS TO PARP INHIBITION

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Replication protein A (RPA) participates in essential protein-protein and protein-DNA interactions that initiate and coordinate DNA replication, repair, recombination, and the DNA damage response (DDR). Small molecule inhibitors have been developed to target each of these interactions. We have previously reported the optimization of RPA inhibitors (RPAi) targeting the protein-DNA interaction and demonstrated anticancer activity alone and in combination with DDR targeted agents as a function of chemical RPA exhaustion in lung cancer models and in a BRCA1 deficient non-cancer retinal pigment epithelial (RPE) model. More recently single-stranded DNA gaps have been implicated in the sensitivity of BRCA1 deficient cancers to poly (ADP-ribose) polymerase inhibitors (PARPi's). We previously have demonstrated that RPAi sensitizes cells to PARPi treatment in BRCA1 wildtype lung cancer models and in a non-cancer BRCA1 deficient retinal pigment epithelial model. The underlying mechanism of combined activity however, was not determined. Here we demonstrate the development of a combined treatment regimen targeting RPA and PARP that abrogates cancer growth in a BRCA1 deficient breast cancer model. These data were recapitulated in a BRCA1 deficient and complemented isogenic UWB1.289 ovarian cancer cell lines. The data demonstrate that in BRCA1 wildtype cancers, similar sensitization still occurs but requires higher concentrations of PARPi. This data is consistent with BRCA1 loss inhibiting gap suppression which is further exacerbated by PARP inhibition. We interrogated the mechanism of RPAi sensitivity and resistance and demonstrate efficacy of repeat dosing suggesting a lack of acquired resistance to RPAi treatment. RPA inhibition had modest effects on S-phase progression and overall cell cycle distribution but live cell imaging reveled that the majority of death was observed as cells progressed through mitosis. Interestingly, the presence of micronuclei was also observed in cells treated with RPAi which were further increased in combination with specific PARPi treatment. These data suggest that RPAi dependent effects in S-phase are perpetuated into mitosis to result in cytotoxic activity.

Basic Science ***Research Technician***

POSTER #83

GENETIC ANCESTRY DEPENDENT VARIABILITY IN STROMAL CELLS: AN UNEXPLORED PLAYER IN BREAST CANCER DISPARITY

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Genetic ancestry dependent variability in cancer incidence, mutation patterns, response to chemotherapy, and outcome has been documented. While an association between social determinants of health and breast cancer disparity has already been established, there is emerging evidence for genetic ancestry dependent variability in normal breast biology impacting breast cancer biology and potentially outcome. Tumor biology studies in the context of genetic ancestry and disparity have often focused on intrinsic properties of tumor cells or tumor infiltrating immune cells. However, other stromal cell types have received very little attention. Through immunohistochemistry of breast tissues from healthy women of African and European ancestry and primary cell culturing system, we had previously demonstrated enrichment of a stromal cell population called PZP cells (PROCR+/ZEB1+/PDGFRA+) with mesenchymal stem-like and fibroadipogenic properties in the normal breast tissues of women of African ancestry. In this study, we used single nuclei ATAC-seq and/or RNA-seq to further characterize stromal fibroblasts in the breast tissues of women of Ashkenazi Jewish-European, European, Indigenous American, Hispanic-European, African, and Asian ancestry. Among eight fibroblast cell clusters generated from women of African and European ancestry, only three cell clusters overlapped between two groups. While Complement Factor D (CFD, also called adipsin) expression was observed in unique fibroblast clusters of African ancestry, the expression of Insulin-like Growth Factor 1 (IGF1) was enriched in clusters unique to European ancestry. Interestingly, previous studies have demonstrated African ancestry-specific genomic variants for CFD linked to cardiometabolic disorders. Additional genes that showed genetic ancestry dependent variability in expression within fibroblasts include ABCA10, ABCA9, ABCA8, NEGR1 (enriched in African ancestry), MMP16, MAGI1, KIAA1217, PTPRK and SEMA5A (enriched in European ancestry). NEGR1 (Neuronal Growth Regulator 1) is a trans-neural growth promoting factor, whereas PTPRK (Protein Tyrosine Phosphatase Receptor Type K) is a negative regulator of EGFR signaling. These results suggest genetic ancestry dependent variability in stromal-epithelial cell communications under normal and cancerous conditions. These genetic ancestry dependent differences could impact intracellular signaling networks in epithelial cells with consequential effects on cancer incidence, mutation patterns, drug sensitivity, and outcome.

Basic Science ***Research associate***

POSTER #84

DIFFERENTIAL RESPONSE OF GCN2 EIF2 KINASE TO SPECIFIC AMINO ACID LIMITATIONS IN PROSTATE CANCER

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Stress response pathways are critical for cellular adaptation and survival to different environmental cues, such as amino acid (AA) starvation. The eIF2 kinase GCN2 is a major sensor of AA in cells, directing transcriptional and translational changes critical for stress adaptation to AA limitations. GCN2 is activated by interaction with uncharged tRNAs, which accumulate during AA limitation. GCN2 phosphorylates the eukaryotic translation initiation factor 2 (eIF2), resulting in repression of global protein synthesis. In parallel, eIF2 phosphorylation also enhances the translation of key mRNA transcripts, such as that encoding the transcription factor ATF4, which directs the transcription of genes involved AA synthesis, transport, and reclamation. Additionally, GCN2 activation decreases cellular AA consumption by reducing translation, altering metabolism, and modulating the cell cycle. Collectively these events are critical for adaptation and survival to AA starvation. Our laboratory has recently shown that GCN2 is activated in prostate cancer (PCa) and is critical for the maintenance of AA homeostasis. We demonstrated in multiple PCa cell lines in culture and *in vivo* models that GCN2 inhibition results in AA limitation and reduced proliferation, showing the importance of GCN2 for PCa growth and progression and its potential as a therapeutic target.

For our studies on the role of GCN2 and its roles in AA homeostasis in PCa, we utilized the androgen-sensitive and castration-resistant cell lines LNCAP and 22Rv1, respectively. We sought to address which amino acid(s) may be limiting to activate GCN2 in the each of the proliferating cell lines. To determine limiting AAs, LNCaP and 22Rv1 cells were cultured in media lacking either a single or multiple amino acids. Our results show that depletion of each of the amino acids resulted in activation of GCN2, increased eIF2 phosphorylation, and enhanced ATF4 expression, as measured by immunoblotting, and decreased global translation. Although GCN2 activity was increased by depletion of any individual AA, we observed that the degree of GCN2 activation and subsequent translation inhibition vary depending on which AA was depleted. Additionally, we investigated how other nutrient sensing pathways, such as mTORC1 or p53, respond to specific AA starvation, and found that depletion of only a subset of AAs sensed by GCN2 result in activation of these pathways. These results suggest that, unlike other nutrient-sensing pathways, GCN2 can sense and respond to limitation for each AA.

Our results highlight the importance of GCN2 in the adaptation and survival of cells to different types of AA limitation. Unlike other nutrient pathways, GCN2 can sense imbalances of any proteogenic AA and coordinate a stress-mediated adaptive response. Determining the mechanistic features for how GCN2 responds to different types of AA starvation will be important to understand its role in health and disease.

Basic Science ***Undergraduate Student***

POSTER #85

CHARACTERIZE THE STRUCTURE-FUNCTION RELATIONSHIP OF ANGIOMOTIN (AMOT) USING SMALL ANGLE X-RAY SCATTERING

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Statement:

This project then has 2 objectives where we use small angle x-ray scattering (SAXS) to determine the structure-function relationships that drive the cellular dysregulation:

1.Identify mutations that cause global structure changes of the ACCH domain structure and radius of gyration (*Rg*).

2.Define the effect of ACCH structural changes on functional ability to reorganize the appearance of lipid phase Bragg peaks in synthetic lipid mixtures.

Based on previous work in this laboratory, we expect the ACCH domain to form rod-like structures due to the forming of oligomers and that these mutations are unable to form these oligomeric states. We also expect that the loss of these oligomers will reduce the functional ability of these mutations to combine lipid phases. The results in the project will improve our understanding how Amots functionally change membranes to promote proliferation and invasiveness of tumors formed from epithelial cells.

Methodology:

Separate out the protein mixtures by size (SEC), determine the approximate molecular weight by mass spectrometry (MALS), and then collect SAXS data on each fraction. Start with a simple analysis of the globular structure of the protein modeled as a sphere. To do this, we look at the Guinier region of the of the SAXS Data.

Use synthetic lipid membranes to characterize changes in the lipid phases present based on the ACCH domain fusing and remodeling the various lamellar phases present in the POPC/POPE/PI 60/20/20 mol% lipid mixture. Use the Bragg peaks present in the scattering patter to determine the d spacing of the various phases present. Bragg peak analysis is used to characterize the lamellar and cubic arrangement of the lipids. Membranes form a lamellar layer at regular intervals where the q-values repeat by integers.

Results:

The ACCH domain mutants (R40T, K49E, R153E, R234G) resulted in larger Rg, as well as radii in comparison to the Wild Type. The Wild Type showed an Rg of 275.5 ± 66.4 Å, and a radius of 398.7 ± 93.9 Å.

The lamellar phase data demonstrates that the wild type got rid of phases, losing peaks 4-8 and 10-11 in the Bragg Peak analysis. Lipid+R234G, lipid+R103G, and lipid+R153E show the mutation has incomplete activity.

Conclusion:

The radius found from this preliminary analysis is larger than what we had previously reported. The data suggests these mutations led to drastic increases in their globular structure, and therefore local residue position & interactions. Through the Guinier analysis of each ACCH domain mutant, the radii of gyration indicate a much larger overall protein model than just the wild type.

Basic Science ***Undergraduate Student***

POSTER #86

AREG DRIVING THERAPEUTIC RESISTANCE IN PDAC

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Pancreatic ductal adenocarcinoma (PDAC) is the third and fourth leading cause of cancer-related deaths in the United States in women and men respectively, and it has the lowest five-year survival rate of all major cancers at only 13%. Though aberrant activation of the small GTPase KRAS is credited as the driving mutation in PDAC, it has been shown that wildtype epidermal growth factor receptor (EGFR) signaling is required for the cancer's initiation and progression. EGFR activation is often achieved through autocrine signaling where the cell produces growth factors which can be processed and released into the extracellular space to interact with EGFR ligand binding domains. PDAC patients variably express the ligand amphiregulin (AREG) on a case-to-case basis. It has been shown that patients with high AREG expression tend to have worse survival compared to patients with low AREG expression, meaning AREG expression has been found to negatively correlate with patient survival. Further, AREG has been implicated in therapeutic resistance in PDAC by driving cellular migration through the mechanism of an epithelial to mesenchymal transition. Currently, EGFR inhibitors are an FDA approved treatment for PDAC patients; however, drug resistance rates are high and the increase in survival is modest. KRAS targeted inhibitors in preclinical trials have also proven ineffective as resistance mechanisms have already been identified. Our lab is currently investigating an alternative therapeutic strategy by indirectly inhibiting downstream oncogenic signals in the EGFR-KRAS pathway. This is achieved by using the small molecule activator DT-061 which stabilizesProtein Phosphatase 2A (PP2A)which targets many KRAS effectors. However, preliminary data in our lab indicatesthat DT-061 may drive the upregulation of AREG expression and shedding to overcome the tumor-suppressive effects of PP2A activation. To investigate this potential mechanism of therapeutic resistance, we have created stable cell lines which overexpress AREG in different human PDAC cell lines. We have measured differential viability and signaling changesbetween cells with AREG overexpression and a control line when treated with DT-061. We have also found a dose-dependent relationship between free AREG concentration in the media and cellular DT-061 sensitivity. Uncovering this relationship between AREG expression and resistance mechanismswould provide critical data for the therapeutic efficacy of this drug in patients with high AREG expression.

Basic Science ***Undergraduate Student***

POSTER #87

UNCOVERING THE MECHANISM FOR REDUCED SURVIVAL IN A NOVEL PDAC MOUSE MODEL WITH PP2A-B56 ACTIVATION

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Pancreatic ductal adenocarcinoma (PDAC) cancer ranks 4th in the leading causes of cancer related deaths in the United States with a 5-year survival rate of just 13%. A driver mutation of PDAC is mutant KRAS, present in ~90% of PDAC patients. For decades, researchers have attempted to target mutant KRAS, and pre-clinical trials for mutant KRAS inhibitors have already shown resistance. Therefore, alternative therapeutic strategies are necessary. Protein phosphatase 2A (PP2A) inhibits many of the downstream effectors of KRAS, and its activation could be a potential therapeutic opportunity.

PP2A is a major serine/threonine phosphatase, composing of a structural “A” subunit, regulatory “B” subunit, and a catalytic “C” subunit. The A, B, and C subunits come together to form the active phosphatase complex. PP2A is known to act as a tumor suppressor in its full active complex. However, little has been done to understand the role of the different isoforms of PP2A, especially with the >15 B subunits, which provide substrate specificity when incorporated into the complex. B56a has been suggested as a subunit that contributes to the tumor suppressive ability of PP2A.

To understand the role of PP2A-B56a in PDAC tumorigenesis, our lab unexpectantly found that both genetic overexpression of the B56a subunit and pharmacological activation of PP2A-B56a in human PDAC cells led to increased oncogenic phenotypes. Subsequent studies have allowed us to build a potential mechanism driving this phenotype, where increased transcription and post-translational processing of epidermal growth factor receptor (EGFR) ligand, amphiregulin, leads to EGFR activation.

Studying the role of PP2A-B56a activation *in vivo* is difficult as overexpressing B56a does not guarantee function or activation into the complex. To circumvent this, the gold standard PDAC mouse model, KPC (KrasG12D: Het, p53R172H: Het, PdxCre1: positive), was crossed with a homozygous deletion of CIP2A (CIP2AHOZ), an endogenous inhibitor of PP2A-B56. Like our *in vitro* human PDAC cell lines, mice withPP2A activation through loss of the inhibitor, CIP2A, had decreased survival compared to the CIP2A wild-type mice.

With our lab’s proposed mechanism for PP2A-B56a activation leading to EGFR ligand release, we sought to uncover the cause for decreased mouse survival in this PDAC mouse model. Cell lines were generated from end stage tumors from the CIP2A wild type (KPC) and CIP2A mutant (KPCC) mice. With these cell lines, we compared the *in vitro* oncogenic phenotypes as well as the cellular mechanisms of each cohort. These ongoing studies promise to deepen our understanding of PP2A-B56a's role in PDAC. More broadly, these findings imply that PP2A and its subunits act in a tissue specific manner.

Basic Science ***Undergraduate Student***

POSTER #88

TOWARD UNDERSTANDING THE ANGIOMOTIN MEMBRANE FUSION ACTIVITIES

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80-90% of all cancers are epithelial cell derived carcinomas. In some of these cancers, overexpression of Angiomotins (Amots) has been found to promote cancer. Amots are a family of adapter proteins that play an important role in the localization and regulation of manyproteins involved in cellular polarity, differentiation, and proliferation. These functions have been accredited to their coiled-coil homology (ACCH) domain's ability to selectively bind, deform, fuse, and reorganize membranes containing phosphatidylinositol (PI) lipids. However, it is unclear how this function leads to tumorigenesis and metastasis. Based on our previous work, we hypothesized that disruption of the ACCH domain's ability to fuse membranes leads to a loss of normal cellular polarization and adhesion, therebyincreasing therates of cellular proliferation and migration (metastasis). To test this hypothesis, we followed up on our previous mutation screens identifying mutations in The Cancer Genome Atlas (TCGA)—gastric adenocarcinoma R153H and endometrial cancer K111H. We present our in vitro approach to understanding the relationship between ACCH domain activity and downstream cellular effects. We use fluorescence microscopy and cellular fractionation to demonstrate how these mutations affect cellular trafficking by measuring Amot80 localization and local concentration of PI lipids within the plasma membrane, Golgi apparatus,ER, and endosomal vesicles. The results provide insight into the role of these individual ACCH domain residuesinmaintaining normal phenotypes, and how their mutation may initiatetumorigenesis.Future work includes measuring the impact of these changes on the cellular proliferation and migration that might indicatemore aggressive cancer phenotypes.

Basic Science ***Undergraduate Student***

POSTER #89

INVESTIGATING THE MECHANISM OF CELL DEATH INDUCED BY THE PHARMACOLOGICAL ACTIVATION OF PP2A IN PANCREATIC DUCTAL ADENOCARCINOMA.

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Pancreatic Ductal Adenocarcinoma(PDAC) is the fourth leading cause of cancer-related death in the United States. KRAS is an oncogene mutated in 90% of PDAC patients. KRAS inhibitors have been discovered but so have resistance mechanisms, highlighting the importance of identifying alternative strategies to target this pathway. PDAC tumour microenvironment evolves with cancer cell progression leading to vascular remodelling and blood vessel collapse. This makes the tumours nutrient deplete. To circumvent this, cells employ KRAS-dependent macropinocytosis-a nutrient scavenging pathway. Macropinocytosis is the process by which cells take up extracellular material by membrane ruffling to form vesicles which then fuse with lysosomes to release nutrients. Targeting KRAS-driven macropinocytosis can be crucial to limiting nutrients to the cell. Protein Phosphatase 2A(PP2A), a heterotrimeric Serine/Threonine phosphatase, is a tumour suppressor and downstream regulator of KRAS.PP2A activity is repressed by mutated KRAS.We have seen that the activation of PP2A with small molecular activator, DT061, prevents macropinosomes from fusing with lysosomes which leads to cell death. But how DT061 regulated PP2A activity affects the macropinocytosis pathway is still unknown. The purpose of this study is to understand the mechanism of cell death caused by the activation of PP2A with DT061. Membrane ruffling is critical for the initiation of macropinocytosis. Rac is a GTPase involved in membrane ruffling. To show that Rac is needed for DT061 mediated macropinocytosis, we used EHT1864, a Rac family inhibitor. When cells were pretreated with EHT1864 prior to DT061, we saw that there is a reduction in the cell death caused by DT061 showing us that Rac is crucial to DT061 mediated cell death. Understanding the pathway of DT061 mediated cell death can be vital to developing this concept as a therapeutic method of treatment for pancreatic cancer patients who currently have the lowest 5-year survival rate(13%) of all cancers.

Basic Science ***Undergraduate Student***

POSTER #90

INVESTIGATING THE DISTINCT INTERACTION OF TGLI1 AND NONO IN GLIOBLASTOMA

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The transcription factor tGLI1 is a truncated splice variant of the known transcription factor GLI1, which plays an important role in the hedgehog signaling pathway. It has been reported that tGLI1 is only expressed in Glioblastoma multiforme (GBM) but is absent in normal tissue. High tGLI1 expression in GBM has been correlated with poor patient survival, highlighting its significance. Characterization studies of tGLI1 have revealed that, despite the loss of 41 amino acids, tGLI1 has retained all known GLI1 functional domains. Previously published literature has also shown that tGLI1 is involved in gene regulation distinct from GLI1. Specifically, tGLI1 binds to and activates genes associated with migration, invasion, angiogenesis, and stemness to promote tumor progression. However, the mechanisms of tGLI1's gain of function are unknown. Our hypothesis is that tGLI1 exhibits distinct transcriptional behavior from GLI1 due to its interaction with specific proteins. To first address our hypothesis, we performed mass spectrometry and identified 30 proteins that interact with tGLI1 and not GLI1. From the list of 30 unique tGLI1 interactions, non-POU domain containing octamer binding protein (NONO) was further studied as it has been shown to alter the binding and activity of transcription factors and elevated expression of NONO has been correlated with reduced patient survival in glioblastoma. GBM cells subjected to co-immunoprecipitation confirmed NONO interacts with tGLI1 to a greater degree than GLI1. To further understand this interaction, we used cellular fractionation to identify where the interaction may be occurring. In cells overexpressing tGLI1, we found a higher concentration of NONO in the nucleus as compared to cells overexpressing GLI1. These studies demonstrate a protein interaction between tGLI1 and NONO that may impact the localization of both proteins. Future studies will further clarify the interaction between tGLI1 and NONO, the relationship of this interaction to GBM phenotypes, and the effect of NONO on the transcriptional activity of tGLI1.

Basic Science ***Undergraduate Student***

POSTER #92

TOWARDS ADVANCING TARGETED DETECTION OF AGGRESSIVE TRIPLE-NEGATIVE BREAST CANCER AND ITS METASTASIS

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Each year in the US, over 100,000 new cases of brain metastasis are diagnosed. Approximately 10-30% of women with aggressive subtypes of Triple Negative Breast Cancer (TNBC) experience metastasis to the brain.TNBC has fewer targeted treatment options than any other type of invasive breast cancer, where treatment generally includes surgery and generalized, non-targeted chemotherapy that has an 80% incomplete response. Therefore, breast cancer brain metastasis (BCBM) has a high mortality rate, particularly due to chemotherapy agents having a limited permeability of the blood brain barrier (BBB). Previous research in the Kimble-Hill lab has shown specific Phosphatidylinositol (PI) to be potential biomarkers for breast cancer aggressiveness. Therefore, we hypothesized that fluorescently labeled antibodies targeting these PI biomarkers can differentiate between co-cultured Neuro 2A cells, a mouse neural crest-derived cell line, and TNBC cell lines. We also hypothesized that intracellular signaling in the culture would result in higher Neuro 2A proliferation and higher TNBC migration rates towards these neuronal cells. Methodology includes fluorescent imaging of the cells constitutively expressing fluorescent tagged proteins and the MTT like CellTitre assay for cell counting within t-tests for statistically significant differences in proliferation and migration rates. We also use Pearson's correlation coefficients to determine the ability to discern the prevalence of PI lipids in each cell population using fluorescent imaging. Our study has therapeutic implications in understanding mechanisms underlying BCBM as well surgical applications for targeted removal of cancerous cells towards an overall goal of improving survival rates.

Basic Science ***Undergraduate Student***

POSTER #93

LIVER METASTASES ACCELERATE CACHEXIA IN MODELS OF LUNG AND PANCREATIC CANCER

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Liver metastases accelerate cachexia in models of lung and pancreatic cancer

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Background: Patients with advanced cancer frequently develop cachexia, a debilitating syndrome characterized by musculoskeletal wasting. Despite affecting most cancer patients, cachexia remains understudied and currently has no cure. The liver represents a common site of metastases and is associated with poor prognosis in patients with lung and pancreatic cancer. We have previously demonstrated that liver metastases (LM) exacerbate cachexia in murine models of colorectal cancer, however, whether LMs heighten musculoskeletal wasting in models of lung and pancreatic cancer is unknown. Here, we aimed to characterize the impact of LMs on musculoskeletal health in commonly used mouse models of lung and pancreatic cancer cachexia.

Methods: C57BL/6J male mice were randomized into groups and enrolled into the lung (study 1) or pancreatic (study 2) cancer study. In study 1, mice underwent intrasplenic (IS) and subcutaneous (SC) injections with saline or LLC cells in one of the following experimental conditions: Sham (IS: saline; SC: saline); low-dose SC (IS: saline; SC: $1.25x10^5$ cells); high-dose SC (IS: saline; SC: $1.0x10^6$ cells); LM (IS: $1.25x10^5$ cells; SC: saline). In study 2, mice underwent IS and orthotopic (OT) injections with saline or T4-KPC cells in one of the following experimental conditions: Sham (IS: saline; OT: saline); OT (IS: saline; OT: $1.0x10^4$ cells); LM (IS: $1.0x10^4$; OT: saline); LM/OT (IS: $0.5x10^4$ cells; OT: $0.5x10^4$ cells). Body weights were monitored throughout the study and *in vivo* plantarflexion torque was assessed to determine changes in muscle function. Upon sacrifice, tissues (muscles and bone) were collected for further analyses.

Results: Animals with lung LMs had reduced fat ($p<0.01$), muscle mass (gastrocnemius: $p<0.01$; quadriceps: $p<0.01$; tibialis anterior: $p<0.001$), and muscle torque ($p<0.05$) compared to all groups. Compared to sham, ultimate force of femur bones was only reduced in mice with LM ($p<0.001$), further suggesting accelerated musculoskeletal impairment. Similar effects were found in animals with pancreatic LMs. When comparing tumor groups to sham, only mice with pancreatic LMs displayed reductions in gastrocnemius ($p<0.01$) and tibialis anterior ($p<0.01$). Similarly, plantarflexion torque was only reduced in mice with pancreatic LMs ($p<0.01$). Molecular characterization reinforced the wasting phenotype, evidenced by elevated atrophy signaling (e.g., STAT3) in animals bearing lung LMs.

Conclusions: Like our prior work in colorectal cancer, these findings suggest that LMs also worsen cachexia in models of lung and pancreatic cancer. To optimize preservation of musculoskeletal health, investigations into the mechanisms by which LMs worsen cachexia are essential.

Basic Science ***Undergraduate Student***

POSTER #94

BRIDGING THE GAP: EXPLORING THE ROLES OF ETS-COACTIVATOR INTERACTIONS IN PROMOTING PROSTATE CANCER

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The ETS family of transcription factors is comprised of 28 members that all share a common C- terminal DNA-binding domain. ETS play essential roles in developmental processes, such as stem cell maintenance, blood vessel formation, and cellular fate. While absent in the normal prostate, four members of the ETS family become expressed in prostate tumors: ETV1, ETV4, ETV5 and ERG. Our lab has previously classified these proteins as *oncogenic ETS* for their ability to promote cellular migration in normal prostate epithelial RWPE1 cells. In order to gain oncogenic function, these ETS transcription factors require interaction with additional proteins. My research explores the interactions between ERG and p53, a tumor suppressor protein, as well as ETV1 and EWS, an RNA binding protein and transcriptional coactivator that promotes ETS activity.

Mutations in p53 occur in nearly half of all cancer cases and promote oncogenesis. Previous studies demonstrate that mutant p53 binds ETS2 at a higher affinity than wildtype p53. This list of interacting ETS partners has been expanded to include ERG. When expressed in RWPE1 cells, overexpression of mutant p53 (R248W), promotes cellular migration in a Transwell system relative to wild type. Introducing a second mutation in p53 (T81A) reverses this phenotype, imitating overexpression of wild type p53. Interestingly, this pattern changes upon evaluation of colony formation. Double mutant p53 drives colony formation at a higher rate than single mutant. In order to evaluate how ERG promotes or changes these observed phenotypes, we aim to repeat these experiments in RWPE1 cells co-transduced for overexpression of both ERG and mutant p53.

Additionally, our lab has established that ETV1, ETV4 and ETV5 form essential interactions with EWS. In prostate tumors, chromosomal rearrangements result in the N-terminal truncations of ETV1, ETV4 and ETV5. We hypothesize that the EWS interaction domain is retained and that these truncated ETVs remain oncogenic. Performing pulldown assays using purified full-length and truncated forms of ETV1 proteins will narrow the potential binding sites. After maintaining stable expression of tETV1 in RWPE1 cells, we aim to evaluate their interactions with EWS, as well as their ability to promote cellular migration and colony formation *in vitro*. Further, immunoprecipitation mass spectrometry will allow for identification of possible co-activator proteins essential for formation of the ETV1-EWS complex.

Continuation of this work hopes to provide critical insight into the molecular mechanisms by which a subset of ETS factors achieve oncogenic function. We further aim to address the contributions of DNA-binding specificity and coactivator interactions in promoting the phenotypes associated with prostate cancer.

Basic Science ***Undergraduate Student***

POSTER #95

THE INFLUENCE OF STROMAL CELL ORIGIN ON CYTOKINE RELEASE IN A SPHEROID MODEL OF ENDOMETRIOSIS AND ENDOMETRIOSIS-ASSOCIATED OVARIAN CANCERS

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Introduction:Endometriosis is a highly prevalent, chronic gynecologic disease in which benign endometrium-like tissues develop outside the uterus, affecting ovaries in 50% of cases. The presence of endometriosis is associated with a higher risk of developing ovarian endometrioid and ovarian clear-cell carcinomas (EnOC and OCCC, respectively), suggesting a unique microenvironment. Endometriotic stromal fibroblasts possess a distinctive proinflammatory transcriptomic profile and drive an inflammatory intraperitoneal climate. The role of benign endometriotic stroma in the regulation of endometriosis-associated ovarian cancer (EAOC) development and progression remains unexplored. We hypothesize that endometriotic stromal cells contribute to the advancement of EAOC viaaltered immune response and cytokine secretion.

Methods:With IRB approval, fresh endometriosis tissues and normal eutopic endometrium were procured and underwent mechanical and enzymatic digestion followed by primary stromal cell isolation.Epithelial cell lines representing benign endometriosis (12z), EnOC (A2780), and OCCC (SKOVip)were co-cultured with the primary endometriotic stromal (EnS) or normal endometrial stromal (NS) cell cultures into spheroids for 72h, and further treated with an endometriosis cytokine cocktail (TNF-α, TGF-β1, IL-1β) or vehicle for additional 24h. Conditioned medium was collected for cytokine quantification via a bead-based Milliplex Human Chemokine Panel(IL-6, IL-8, MCP-1, PAI-1)immunoassay coupled with a Luminex platform.

Results:In comparison to co-culture with NS, benign 12z/EnSspheroids exhibited most prominent growthin cytokine production (IL-6 – 7-fold; IL-8 – 6-fold; PAI-1 – 2.5-fold; $n \geq 3$; $p < 0.001$). Malignant OCCC SKOVip/EnS co-culture resulted in a 4-fold increase in IL-6, a 2-fold increase in IL-8, and a 5-fold drop in MCP-1 levels as compared to SKOVip/NS controls ($n \geq 3$; $p < 0.001$). Malignant EnOC A2780/EnS spheroids resulted in a slight, 1.5-fold elevation of IL-6and a 1.4-2.5-fold decrease in other cytokines($n \geq 3$; $p < 0.05$).Importantly, the presence of EnS in co-cultures was consistent with attenuated cytokine secretion response to stimulation by the immune cocktail across all tissue types ($n \geq 3$; $p < 0.05$).

Conclusion:Our workhighlighted the distinct baseline inflammatory mediator secretion andthe altered response to additional immune pressure by ovarian malignant tissues within the endometriotic stromal environment.Identifying cytokine production based on the origin or disease state of stroma – and its interaction with epithelial cells – can further the understanding of the tumor microenvironment for EAOC and identify molecular targets for disease prevention and therapy.

Basic Science ***Undergraduate Student***

POSTER #96

ACTIVE MUSIC ENGAGEMENT TO REDUCE TRAUMATIC STRESS SYMPTOMS AND IMPROVE WELL-BEING IN PARENTS OF YOUNG CHILDREN WITH CANCER: HOW DOES IT WORK & WHO BENEFITS?

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Background/Purpose: Mechanistic trials help us understand how intervention programs work and better identify individuals who may benefit. This has important implications for evidence-informed care and referral systems. The Active Music Engagement (AME) intervention uses music-based play to mitigate the interrelated distress experienced by parents/children during cancer treatment. The purpose of our trial (R01NR1578) was to identify mediators and moderators of AME effects on parent/child outcomes using quantitative and qualitative methods.

Methods: Child/parent dyads (n=125) were randomized to AME or attention control. Each group received three sessions with a credentialed music therapist for three consecutive days with data collection at baseline, post-intervention (T2), and 30-days later (T3). Potential proximal mediators included within session child and parent engagement. Potential distal mediators included changes in perceived family normalcy, parent self-efficacy, and independent use of play materials. Potential moderators included parent/child distress with prior hospitalizations, parent traumatic stress screener (PCL-6), and child age. Outcomes included child emotional distress and quality of life; parent emotion, traumatic stress symptoms (IES-R), well-being; and family function. Mediation effects were estimated using ANCOVA, with indirect effects estimated using the percentile bootstrap approach. Moderation effects were tested by including appropriate interaction terms in models. We also conducted semi-structured interviews with a subset of AME parents (n=28).

Results: No significant mediation effects were observed. Child distress with prior hospitalizations moderated AME effects for IES-R intrusion subscale scores at T2 ($p=0.01$) and avoidance subscale scores at T3 ($p=0.007$). Traumatic stress screener scores (PCL-6) moderated intervention effects for IES-R hyperarousal subscale scores at T2 ($p=0.01$). Qualitative findings included four themes that explain how AME counteracts stress for parents/children including cognitive reappraisal.

Conclusions/Implications: AME mitigates traumatic stress symptoms and supports well-being in parents of children with cancer, particularly parents who screen high for traumatic stress and whose children are more highly distressed with hospitalization.

Behavioral ***Faculty***

POSTER #97

A QUALITATIVE EXPLORATION OF FEAR OF RECURRENCE IMPACT AND COPING AMONG BREAST CANCER SURVIVORS PARTICIPATING IN A RANDOMIZED CONTROLLED TRIAL

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Background: With an overall five-year survival rate of 91%, many of the 4.1 million breast cancer survivors (BCS) in the United States experience fear of cancer recurrence (FCR) at levels warranting psychosocial care. Approximately 28% of BCS report moderate FCR and 22% report high levels of FCR. These clinical levels of FCR often negatively impact quality of life. Identifying the specific nature of FCR and its impact on BCS' lives can inform intervention development.

Objective: We analyzed qualitative data for response themes from BCS enrolled in an FCR intervention trial regarding the impact of FCR on daily life, how BCS coped with FCR prior to intervention, and what they hoped to gain from trial participation. We then examined response variation by BCS' baseline FCR score.

Method: This qualitative study was part of a randomized controlled trial comparing three behavioral interventions for BCS reporting clinical FCR (FCR-7 score ≥ 17) at enrollment. BCS (*N*=384) completed a baseline survey including three open-ended questions regarding their FCR experiences. Responses were analyzed by a team of four coders using content and thematic analysis. Coded themes were sorted by baseline FCR-7 scores to search for patterns in thematic categories by FCR level.

Results: Across the sample of BCS, 81.8% were White, the mean age was 55.8 (*SD*=12.0) years, and 55.7% were within 2 years of completing cancer treatment. The average baseline FCR-7 score was 21.5 (*SD*=6.9). Qualitative responses revealed five daily FCR impact domains: emotional, behavioral, cognitive, relational, and professional. BCS with high FCR-7 scores (≥27) reported impact in all five domains. Moderate FCR-7 scores (17-26) and minimal FCR-7 scores (6-16) reported intermittent impact or impact in one domain. Prior to intervention, most BCS reported use of at least one coping strategy, finding it somewhat helpful. Coping strategies were consistent across all FCR-7 score levels and fell under five key domains: behavioral, emotional, cognitive, social, and spiritual. However, the number of strategies increased and tended toward avoidant coping with higher FCR-7 scores. BCS predominately identified a desire to gain more effective coping strategies from study participation. As FCR-7 scores increased, so did the number of reasons for study participation. (Illustrative quotes from BCS for all themes will be woven into the conference poster presentation.)

Conclusion: Results suggest many BCS with clinical FCR are suffering without adequate means of coping. Clinicians are encouraged to regularly ask BCS about FCR and its impact, as the discussion can lead to a better understanding of BCS' emotional well-being and potential intervention recommendations. Further research is needed to determine which behavioral interventions are most effective in supporting BCS in coping adaptively with FCR.

Behavioral ***Graduate Student***

POSTER #98

RACIAL DISPARITIES IN OBESITY AND RELATED MULTIMORBIDITY AMONG BREAST CANCER SURVIVORS

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Background. Breast cancer survivors (BCS) report greater multimorbidity (i.e., co-occurring medical conditions) compared to other cancer survivors. Common multimorbidities among BCS include obesity and associated conditions such as hypertension, pain, asthma, and osteoarthritis. Unfortunately, multimorbidity is dangerous and adversely impacts all stages of breast cancer care and treatment, even before diagnosis. Multimorbidity is linked to greater symptom burden, functional decline, and lower adherence to surveillance. Critically, multimorbidity may also disproportionately impact Black BCS, is attributed to higher obesity rates, and is putting this community at even higher risk of adverse outcomes than other racial groups. The objective of this study is to compare prevalence rates of obesity and associated multimorbid conditions across the different racial groups.

Methods. Participants were 384 female BCS (314 White, 56 Black or African American, 12 Other/Multiracial; mean age 55.8 [SD = 12.0] years) enrolled in the FACing Fear randomized controlled trial (*R01CA230542*; PI Johns; Clinical Trials Identifier *NCT05364450*). Frequencies of reported multimorbid conditions (i.e., obesity, psychiatric conditions, cardiometabolic disease, allergies, liver or kidney disease, chronic pain, gastrointestinal disease, neurological disease, anemia, and trauma history) were calculated across the three racial categories: white, Black or African American, and Other/Multiracial. ANOVA, Kruskal-Wallis, pairwise Wilcoxon, Chi-squared and Fisher's exact tests were performed as appropriate to determine significant differences in percentages across racial groups.

Results. 64.8% of Black BCS vs. 42.4% of white BCS vs. 16.7% of other/multiracial exhibited obesity (BMI median (IQR) = 32.6 (27.5 ~ 40.7) kg/m^2 vs. 28.5 (24.7 ~ 34.9) kg/m^2 vs. 24.6 (20.7 ~ 26.0) kg/m^2, respectively, $p<0.0001$). Adjusted by multiple comparisons, median BMI was significantly larger in black BCS as compared to white BCS ($p=0.0005$) and other/multiracial BCS ($p=0.0012$). Black BCS also reported more obesity-related comorbid conditions than white and other/multiracial BCS, such that Black BCS had highest rates of hypertension (57.1% vs. 34.4% and 25.0%, $p=0.004$), arthritis (42.9% vs. 25.8% and 8.3%, $p=0.010$) and anemia (12.5% vs. 4.1% and 0.0%, $p=0.045$), highest levels of pain (4.0 (1.0 ~ 6.0) vs. 2.0 (1.0 ~ 4.0) and 2.0 (1.0 ~ 4.0), $p=0.014$) and traumatic experiences (28.5 (20.0 ~ 47.0) vs. 21.0 (12.0 ~ 32.0) and 21.0 (12.0 ~ 26.5), $p<0.000$) among the three racial groups of BCS.

Conclusions. Black BCS had greater multimorbidity for certain conditions. Specifically, Black BCS in this sample had higher rates of obesity and multiple obesity-related issues (i.e., hypertension, arthritis, and anemia as well as higher pain and trauma levels). Critically, obesity predicts higher mortality among Black BCS and has been identified as contributing to other multimorbid conditions, delayed diagnosis, and suboptimal treatment outcomes. These findings have implications for effective obesity treatment in the context of cancer survivorship.

Behavioral ***Graduate Student***

POSTER #99

VALIDATION OF THE ABBREVIATED PTSD CHECKLIST- CIVILIAN AS A TRAUMATIC STRESS SCREENER FOR PARENTS OF CHILDREN WITH CANCER

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Background: Parents of children with cancer often experience traumatic stress symptoms (TSS) associated with their child's diagnosis and treatment. For some, TSS will diminish over the course of treatment and remission. For others, TSS remains prevalent and severe requiring intervention. The Standards of Psychosocial Care of Children with Cancer and Their Families states that parents of children with cancer should have early assessment of their mental health needs and access to intervention. The availability of brief screeners validated for parents of children with cancer is critical to meeting this standard. There are full assessments of psychosocial risk however, there is an absence of validated short screeners for TSS. The PCL-6 would allow for a quick assessment of TSS, enabling clinicians to easily identify parents in need of specialized services.

Methods: This secondary analysis used data collected during a multisite trial examining an intervention for young children (3-8 years old) with cancer and their parents. This dataset included parental self-report measures of traumatic stress (PCL-6; IES-R), mood disturbance (POMS), and wellbeing (Index of Wellbeing).

Sample. 136 parents of young children undergoing active treatment for cancer at four COG hospitals.

Analysis. For construct validity, Spearman's correlation coefficient was calculated for baseline scores of the PCL-6 with baseline scores for the IES-R, POMS-SF, and Index of Wellbeing.

Results: Results indicate good convergent construct validity for the PCL-6 with the IES-R (Spearman's rho=.656 ($p > .001$, 95% CI [.545, .745]) and POMS-SF (Spearman's rho= .733 ($p > .001$, 95% CI [.641, .804]); reasonable divergent construct validity for the PCL-6 with the IWB, (Spearman's rho= -.416 ($p > .001$, 95% CI [-.550, - .262]).

Implications: These tests confirmed that the PCL-6 is a valid brief screening assessment that can be used to improve the tailored delivery of supportive psychosocial interventions for parents of children with cancer.

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

POSTER #100

ACCEPTANCE AND COMMITMENT THERAPY REDUCES FATIGUE AND SLEEP INTERFERENCE IN WOMEN WITH METASTATIC BREAST CANCER

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Background: Fatigue is a persistent and debilitating symptom in metastatic breast cancer (MBC) patients, over 50% of whom report moderate-to-severe fatigue. Addressing fatigue and sleep interference with activities, mood, and cognition is critical to improving the quality of life of this population. Acceptance and Commitment Therapy (ACT) is a behavioral intervention that has shown promise for reducing symptom-related suffering in cancer patients. Rather than aiming to reduce symptom severity, the goal of ACT is to increase psychological flexibility so that difficult internal experiences (e.g., physical symptoms, feelings, thoughts) interfere less with meaningful activities.

Purpose: This NCI-funded Phase II randomized controlled trial (RCT) examined the efficacy of telephone-based ACT in reducing fatigue and sleep interference with functioning in women with MBC.

Methods: A total of 250 women with MBC who had moderate-to-severe fatigue interference were recruited from hospitals in Indiana and Illinois and randomly assigned to either the ACT intervention or an education/support control condition. Women in both conditions attended six weekly 50-min telephone sessions. Outcomes were assessed at baseline and at 2 weeks, 3 months, and 6 months post-intervention. The primary outcome was the Fatigue Interference subscale of the Fatigue Symptom Inventory. In addition, sleep interference was assessed with the Patient-Reported Outcomes Measurement Information System (PROMIS) sleep-related impairment measure. Data were analyzed using linear mixed models with an unstructured covariance structure and multiple imputation. The model included the main effects of time (as categorical) and study group and the time-by-study group interaction. A treatment effect was evidenced by a significant time-by-study group interaction at $p<.05$.

Results: Mixed-model analyses yielded significant time-by-study group interaction effects for both fatigue interference ($F=3.18$, $p=0.025$) and sleep interference ($F=3.42$, $p=0.018$). Mean scores for fatigue interference declined over time for the ACT group (3.93, SD=1.80 at baseline to 3.13, SD=2.29 at six months), while there was minimal decline for the education/support group (3.87, SD=1.83 at baseline to 3.68, SD=2.12 at six months). Mean scores for sleep interference also declined for the ACT group (56.48, SD=7.08 at baseline to 53.52, SD=7.99 at six months) and were relatively stable for the education/support group (54.77, SD=8.23 at baseline to 54.42, SD=8.46 at six months).

Conclusion: Our ACT intervention shows evidence of efficacy in reducing fatigue and sleep interference with functioning in MBC patients. Given widely available training in ACT, it can be disseminated to clinicians who care for this population.

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

POSTER #101

REMOVING BARRIERS TO COLORECTAL CANCER SCREENING IN RURAL INDIANA: IT TAKES A VILLAGE

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Background: Uptake of colorectal cancer (CRC) screening in rural Indiana is lower than the state as a whole. In collaboration with the Indiana Rural Health Association, we have initiated a National Cancer Institute-funded implementation study (R01CA258557) to improve uptake of screening. The study involves an evidence-based multicomponent intervention (EBI) in 11 rural health clinics clustered in 3 groups within southeast, southwest, and east central Indiana. The EBI will support the clinics in addressing access, patient knowledge about screening, patient navigation, and provider recommendations. A significant barrier to screening in rural Indiana is cost, which affects uninsured and underinsured persons. Here, we describe how we are addressing some of the financial barriers to screening.

Methods: We will evaluate the effectiveness of a bundled implementation strategy to increase uptake of EBIs for CRC screening. The Promoting Action on Research Implementation in Health Services (iPARIHS) framework will guide assessment of contextual factors including innovation/evidence, recipient/individual, and inner and outer context. To leverage external resources, our study team involved corporate partners, healthcare systems, and the CheckIt4Andretti Foundation, regarding provision of resources for uninsured/underinsured rural Indiana residents.

Results to date: Our clinical partners, the pharmaceutical/device industry and a foundation have expressed support for implementation of rural CRC screening, including the removal of financial barriers. Polymedco will donate fecal immunochemical tests, Exact Sciences will donate mt-sDNA tests, and Braintree Laboratories will donate liquid and tablet low-volume colonoscopy preparation. Most significantly, the CheckIt4Andretti Foundation will underwrite the colonoscopy facility fee for health care systems willing to accept a structured payment and if endoscopists will donate their effort. Three health care systems have agreed to work with the Andretti foundation to provide colonoscopy services to the underinsured or uninsured, including the Margaret Mary Health system (southeast IN), Adams Health system (east central Indiana), and Deaconess-Gibson Health system (southwest IN). At least one endoscopist at each site has agreed to donate effort.

Conclusions: Some of the barriers to screening in rural settings are financial. As we implement this NCI-funded study to improve uptake of screening, we have formed a project coalition of academia, industry, healthcare systems, physician group practices, and a non-profit foundation. We envision building on this model of cooperation and generosity to establish a state-wide program to mitigate or remove some of the financial barriers to screening and hope it will provide a model for expansion in other settings of need.

Community-directed research poster ***Faculty***

POSTER #102

ASSOCIATION BETWEEN HEALTH INSURANCE COVERAGE AND STAGE OF DIAGNOSIS FOR CERVICAL CANCER AMONG WOMEN IN INDIANA FROM 2011-2019

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Introduction – Cervical cancer is one of the most common types of cancer among women and it is caused by infection from the human papillomavirus (HPV). The incidence rates of cervical cancer have increased in Indiana from 7.8 in 2008-2012 to 8.4 in 2013-2017. In 2020, the screening rate for cervical cancer among women in Indiana was 76% when compared to a national average of 78%. Health insurance status is an important determinant for health outcomes for patients with cancer. This study aims to assess the extent to which health insurance coverage is a contributing factor to the stage of cervical cancer diagnosis.

Methods – We examined reported cases of cervical cancer among women (N=2518) using cervical cancer registry data from the Indiana State Department of Health from 2011-2019. Using multinomial logistic regression model, we examined the associations of both race/ethnicity and insurance status with stage of diagnosis after adjusting for age at diagnosis.

Results – In this sample, the average age at diagnosis was 48.98 years (SD = 14.98). The largest proportion of the sample was between 45 to 64 years of age (41.7%), Non-Hispanic White (85.5%), had private insurance coverage (41.8%), had cervix uteri, NOS as the primary site of diagnosis (76.5%), lived in a metropolitan area (74.5%) and had squamous cell carcinoma (73.4%). The largest percentage of Non-Hispanic White patients had private insurance (50.9%), whereas the largest percentage of Black patients had Medicaid (39.3%). 20.2% of Hispanic patients were uninsured when compared to 7.7% Non-Hispanic White and 3.6% Black patients. The multivariate analysis shows that women who are uninsured (OR = 2.475) and those who have Medicaid (OR = 2.321) were significantly more likely to be diagnosed at the regional stage than the in-situ stage compared to women with private insurance. Additionally, women who are uninsured (OR = 4.432) and those who have Medicaid (OR = 3.007) were significantly more likely to be diagnosed at the distant stage than in-situ, compared to women with private insurance.

Conclusion – The findings show that insurance status is associated with the stage of diagnosis for cervical cancer and detection at regional or distant stages often leads to higher morbidity.

Impact – Increased coverage for routine cervical cancer screening and preventive care services is recommended, especially for uninsured women and women with public insurance such as Medicaid or Medicare.

Community-directed research poster ***Graduate Student***

POSTER #103

PHASE I IMMUNOTHERAPY TRIAL TESTING OF TWO HER-2 PEPTIDE VACCINES (B-VAXX) IN PATIENTS WITH ADVANCED SOLID TUMORS.

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Purpose: Phase I study (NCT 01417546) evaluated the safety, optimal dosage, and immunogenicity of the B-Vaxx vaccine. The first component mimics the binding area of trastuzumab and the second mimics pertuzumab' s binding area. With this vaccine we hope to elicit a robust antibody response reacting similar or better than therapy combining these two Anti-HER-2 antibodies. Both trastuzumab and pertuzumab have been approved for use in patients. It has been found that patients treated with these antibody therapies develop resistance to treatment. We have tested a whole new methodology that will focus on patients making their own antibodies to HER-2 that will act over time to fight their cancer.

Patients and Methods: The vaccine is composed of two types of peptide sequences. One type that acts as a target for B-cells, the cells in the body that make antibodies; and the other part reacts with T-cells which help to mature the B-cells and help them make high levels of therapeutic antibodies. The two peptides were dissolved and mixed with adjuvant which is a component that is given with most vaccines to prime the immune system and deliver the vaccine over time. To complete the protocol a patient had to receive a primary vaccine and a boost at 3 weeks and 6 weeks after the first treatment.

Results: 49 Patents entered the study, all but two had receive multiple rounds of chemotherapy averaging 4 rounds of treatment before starting our therapy. Twenty-eight patients received all three treatments. 6 stage 4 cancer patients survived long enough to receive a six-month boost and one patient received 7 six-month boosts. No serious adverse reactions or dose-limiting toxicities were observed in any of the patients. The vaccine treatments were well tolerated. Of all the patients receiving at least 1 dose, 24% showed light or moderate reaction at the vaccination site not needing any treatment. Including all of the patients that received at least 1 treatment, 2 patients had partial responses to the therapy, 14 had a stabilization of disease and 19 of the patients had a progression of their condition.

Conclusions: The combination vaccine is safe, exhibits antitumor activity, and shows early indications that peptide vaccination may avoid therapeutic resistance and offer a promising alternative to monoclonal antibody therapies.

Future developments: We plan to start a new Phase 1 clinical trial study at the Simon Comprehensive Cancer Center testing the effectiveness of B-Vaxx treatment in cancer patients that have not had extensive chemotherapy which may limit the effectiveness of vaccine therapy. The study will be enrolling patients Breast and Colorectal cancers (CRC) with overexpression of the HER-2/EGFR tumor markers. This should allow for the best evaluation of vaccine therapy.

Community-directed research poster ***Research Technician***

POSTER #104

REFINING STRUCTURE OF THE AMOT COILED COIL HOMOLOGY DOMAIN TO UNDERSTAND FUNCTION IN EPITHELIAL CELL CANCERS

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1 in 8 women in the United States will develop breast cancer in her lifetime. Epithelial cell cancers result from the overexpressionof Angiomotins (Amot). Amots are a family of adaptor proteins that control the location of proteins responsible for cell proliferation, migration, and maintenance of normal cell structure. This trafficking function is related to the ability of Amot's coiled coil homology(ACCH) domain to associate with and remodel cellular membranes. We are interested in deciphering the structure of the ACCH domain to better understand this regulatory function.Our lab's previous research proposeda theoretical structure of the ACCH. This project focuses on refiningthis structure, usingmodeling tools like RoseTTAFold, trRosetta, and GoogleCoLab, against globular envelope dimensions determined experimentally with small-angle X-ray scattering(SAXS). Approximately 80 modelswere generated and comparedto current SAXS data. We also investigated mutations of theACCH to better understand how single-amino acid changes affect structure and therefore functionality. Specific focus was onArg-153mutations and how structural differences cause previously documented reductions in association with cellular membranes. Refined structures are key tounderstanding how all documented Amotmutations lead to cancer initiation and metastasisto find effective cancer therapeutics.

Community-directed research poster ***Undergraduate Student***

POSTER #105

THE RELATIONSHIP BETWEEN RURAL RESIDENCE AND CERVICAL CANCER SCREENING IN THREE SUB-SAHARAN COUNTRIES WITH DIFFERENT NATIONAL SCREENING POLICIES

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PURPOSE: Very few sub-Saharan African (SSA)countries have implemented a systematic cervical cancer screening program, and the screening rates remain very low across countries. Even lower rates are observed in rural compared to urban settings within studied countries, but the association between place of residence and cervical cancer screening uptake in SSA countries is understudied. We examined cervical cancer screening uptake between urban and rural women aged 30-49 years in three SSA countries chosen by their country-specific screening strategy (Burkina Faso, with a systematic population-based cervical cancer screening program; Tanzania with opportunistic screening options only but a systematic Human Papilloma Virus vaccination program; and Ghana, which has implemented neither one).

METHODS: We used Demographic and Health Survey data from three SSA countries, Burkina Faso, Ghana, and Tanzania, including women aged 30-49 eligible for cervical cancer screening, categorized by place of residence as urban or rural. Using country-specific survey design weights, we described other participant sociodemographic characteristics, then estimated through logistic regression the crude and adjusted association between place of residence and cervical cancer screening in six models.

RESULTS: Rural participants represented 69.5% in Burkina Faso, 64.6% in Tanzania, and 42.8% in Ghana. Burkina Faso women reported higher cervical cancer screening prevalence at 19.9%, and Ghana participants the lowest at 7.4%. However, with a 12.2% screening uptake, Tanzania showed a wider (13.1%) divide in rate difference between urban and rural populations. In the crude model, compared to urban participants, rural women were 48% less likely to be screened for cervical cancer in in Burkina Faso (OR 0.52 [95%CI 0.43, 0.63]), 62% in Ghana (OR 0.38 [95%CI 0.29, 0.51]) and 69% in Tanzania (OR 0.31 [95%CI 0.25, 0.39]). In the adjusted model, rural women remained 45% and 66% less likely to be screened for cervical cancer in Ghana (aOR 0.55 [95%CI 0.40, 0.76]) and Tanzania (aOR 0.44 [95%CI 0.34, 0.57]), respectively, but those in Burkina were equally likely to get screened (aOR 0.96 [95%CI 0.76, 1.21]).

CONCLUSION: In a SSA country with an existing systematic screening program and with ongoing efforts to increase cervical cancer screening coverage, like Burkina Faso, it is possible to minimize or close the gap in the odds of getting screened between urban and rural populations. Converging strategies are needed to make screening services available and accessible to the rural population. Further screening uptake studies are needed on this topic with more data.

Keywords: cervical cancer screening, screening policy, sub-Saharan Africa, global health

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

POSTER #106

UNSUPERVISED CLUSTERING OF MORPHOLOGY PATTERNS ON WHOLE SLIDE IMAGES GUIDE PROGNOSTIC STRATIFICATION OF GLIOBLASTOMA PATIENTS

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BACKGROUND

Glioblastoma is the most common malignant adult brain tumor with a poor prognosis and heterogeneous morphology. Stratifying glioblastoma patients according to overall survival (OS) from H&E-stained histopathology whole slide images (WSI) using advanced computational methods is a challenging task with direct clinical implications. We hypothesize that quantifying morphology patterns present in WSI can yield biomarkers of prognostic relevance contributing to optimizing clinical decision-making.

MATERIAL AND METHODS

This work is based on 188 glioblastoma (IDH-wt, grade 4) cases identified in the TCGA-GBM and TCGA-LGG, based on the 2021 WHO classification criteria. One diagnostic WSI from each patient at 10X magnification was selected ($n_{training}/n_{hold\text{-}out\text{-}test}$ =152 /36) and labeled as short (<9 months, n=94) or long (>13 months, n=94) survivors. The WSI is split into non-overlapping 256x256 patches, and extensive patch-level WSI curation is conducted to discard artifactual content, i.e., glass reflections, pen markings, black lines on the slide, and tissue tearing. Each patch is described by a feature vector of 512 dimensions using a pre-trained VGG16. Principal component analysis reduced the dimensionality of these vectors to 236 such that 85% variance is retained. Unsupervised k-means clustering revealed distinct groups of morphology patterns, where the number of clusters is automatically determined based on the rand index and silhouette coefficient. The proportions of these patterns describe the tumor's spatial heterogeneity and are used to distinguish between short and long survivors using a random forest classifier.

RESULTS

We identified seven clusters of distinct morphology patterns, including 3 categories of tumor cellularity (low/intermediate/high), necrosis/macrophages, and other prognostically-relevant characteristics. The patient-level short vs long survivor prediction is driven by the proportion of these patterns present in WSI achieving classification accuracy, sensitivity and specificity of 83.83%, 94.44%, and 72.72% respectively on the hold-out test set. The high sensitivity signifies the algorithm's ability to accurately predict the short survivors which is important in treatment planning and patient management.

CONCLUSION

The interpretability analysis of various morphologic patterns in distinct histologic sub-regions of glioblastoma may uncover correlations with short and long survivors, providing a deeper understanding of the complex relationship between histologic features and clinical outcomes and shedding light on the heterogeneity of glioblastoma. This association could offer additional prognostic information to clinical neuropathologists during microscopic assessment, leading to more refined prognostication and potentially impacting clinical decision-making for improved patient outcomes.

Translational/Clinical Research ***Faculty***

POSTER #107

THE COMPREHENSIVE OPEN FEDERATED ECOSYSTEM (COFE): ENABLINGIMPACTFUL HEALTHCARE STUDIES

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Purpose:

Robustness & generalizability of artificial intelligent (AI) models is dependent on using ample &diverse data. Data ownership & legal hurdles hinderaccess to such data in the current paradigm of multi-sitecollaborations. To address these, we introduce the community-driven Comprehensive Open Federated Ecosystem (COFE), which intends to make distributed healthcare AI more accessible by leveraging zero/low code principles. COFE models get to learn across data silos using federated learning (FL) & we assess their performance on diverse out-of-distribution datasets using federated evaluation (FE). As initial use-cases, COFE enabled the Federated Tumor Segmentation (FeTS) Initiative,which focused on the boundary detection of histologically-distinct brain tumor sub-compartments in MRIs cans, & the FeTS Challenge, the first FL-focused community benchmark.

Methods and Materials:

We designed COFE to be agnostic to data type (imaging, EHR, genomics) & themodel to train. COFE offered an end-to-end solution to the FeTS initiative through 4 distinct modes of operation: data preprocessing, automatic tumor sub-compartment delineation by label fusion of pre-trained AI models, manual refinement of these delineations & FL using the Linux Foundation's OpenFL. Additionally, building upon the collaborative network of the FeTS initiative we used MedPerf to conduct the FeTS Challenge, which followed a clinical trial design to evaluate AI methods on real-world conditions.

Results:

In the FeTS Initiative, we observe an average improvement of 25% in the accuracy of the model on each collaborator's validation data when compared to a model trained on publicly available data. 71 institutions with more than 6,000 subjects diagnosed with Brain Tumors were part of FeTS initiative study. Notably, the difficult regions of the tumor showed the most improvement, with the most prominent being the region for surgical intervention, the tumor core, which improved by 33%. In the FeTS Challenge, inference-time data augmentation combined with post-processing yielded optimal results.

Conclusions:

Our findings on FeTS support that COFE enables training of a consensus AI model that has gained knowledge from data of geographically distinct collaborators, while the data of each collaborator are always retained within their institution, overcoming legal & ownership hurdles. At the same time, it can perform evaluations of pretrained models across completely out-of-distribution datasets to quantify the robustness & generalizability of the model.

Clinical Relevance/Application:

Federated Learning allows training of AI models using diverse datasets without sharing the data, while Federated Evaluation allows AI models to be trained on true out-of-sample data. Combined, this would result in more robust models without raising ethical dilemmas.

Translational/Clinical Research ***Faculty***

POSTER #108

UNRAVELING THE METABOLIC ACHILLES' HEEL OF PANCREATIC DUCTAL ADENOCARCINOMA: TARGETING REF-1 AND BEYOND

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Treating pancreatic ductal adenocarcinoma (PDAC) remains exceptionally difficult, with scant advancements in clinical management over the last thirty years. Aptly dubbed the "graveyard of chemotherapy," PDAC poses formidable clinical challenges. Even the most effective first-line chemotherapy regimens merely extend median survival for patients with metastatic disease beyond 12 months. Due to its poorly vascularized and fibrotic environment, tumor cells encounter hypoxia and nutrient scarcity and hence adapt metabolically by engaging processes like autophagy and macropinocytosis. Key genes such as HIF1A, KRAS, and MYC help PDAC reshape tumor metabolism favoring glycolysis thereby, promoting metastasis and therapy resistance. Targeting these cancer-specific metabolic genes offer a promising approach to overcome PDAC's inherent and acquired chemoresistance. Redox effector factor-1 (Ref-1) activates various transcription factors through its redox activity, including HIF-1a, contributing significantly to metabolic reprogramming and altered gene expression in PDAC. We recently published Ref-1's redox function to be crucial for mitochondrial metabolic regulation, and that inhibition of Ref-1 suppresses TCA cycle, Oxidative phosphorylation (OXPHOS) and tumor growth and metastasis in PDAC models. As further proof of Ref-1's role in regulation of OXPHOS, PDAC cells are more sensitive to Ref-1 inhibitors in galactose-containing media compared to glucose. Therefore, our hypothesis is that Ref-1 inhibitor efficacy can be increased through identification of additional tumor-specific metabolic targets that induce synthetic lethality when combined with Ref-1 inhibition. To identify Ref-1 synergistic metabolic targets, two approaches were used: global metabolomics profiling (Pa03C) and a metabolism-centered CRISPR/Cas9 KO library screening (MiaPaCa2) after treatment with APX2014, a potent Ref-1 redox inhibitor. The CRISPR screen identified several interesting synthetic lethal genes for combination with Ref-1 redox inhibition, including PRDX1, G6PD, and SEPHS2. Further, integration of the global metabolomics profile with our published scRNA seq data shows significant changes in both substrates and enzyme gene expression of multiple metabolic pathways, with predicted decrease in TCA cycle and nucleotide biosynthesis, and increased glutaminolysis following Ref-1 inhibition. Ongoing studies include prediction of combinatorial lethality enabled by co-suppression of Ref-1 and other metabolic targets and then, validation using our *in vitro* and *in vivo* model of PDAC PDX tumor lines co-cultured with matched cancer-associated fibroblasts.

Translational/Clinical Research Faculty

POSTER #109

COMBINATION IMMUNOTHERAPY WITH B-CELL EPITOPE PEPTIDE VACCINE (PDL1-VAXX, PD1-VAXX AND CTLA4-VAXX) SHOWS EFFECTIVE ANTI-CANCER IMMUNITY IN MULTIPLE SYNGENEIC MICE MODELS

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Inhibition of checkpoint receptors with monoclonal antibodies targeting CTLA-4 and PD-1/PD-L1 has shown dramatic clinical success in many types of cancer. However, many patients exhibit treatment resistance and immune-related adverse events (irAEs). Monotherapy targeting PD-1/PD-L1 axis or CTLA-4 pathway show limited efficacy. Novel therapeutic strategies and combination agents are urgently needed to improve the treatment efficacy in cancer patients. We have developed PD1-Vaxx, PDL1-Vaxx and CTLA4-Vaxx peptide vaccines as novel optional immunotherapy strategies versus monoclonal antibody treatment Monotherapy with PD1-Vaxx, PDL1-Vaxx and CTLA4-Vaxx have shown effective inhibition of tumor growth and to extend survival rates in multiple BALB/c (CT26, 4T1 and D2F2) and C57BL/6J (MC38 and B16-F10) syngeneic tumor models. We have also shown that combination immunotherapy of checkpoint inhibitors can improve anti-tumor efficacy as well as enhancing survival rates. The mice immunized with peptide vaccines elicit high titers of polyclonal antibodies with different subtypes (IgG1, IgG2a, IgG2b and IgG3). We will describe our studies of combination immunotherapy with B-cell epitope peptide vaccines, including PD1-Vaxx plus HER-2 vaccine (B-Vaxx), PDL1-Vaxx plus B-Vaxx, PD1-Vaxx plus PDL1-Vaxx, and PDL1-Vaxx or PD1-Vaxx plus CTLA4-Vaxx in several BALB/c syngeneic cancer models. The results indicate combination immunotherapy with B-cell peptide cancer vaccine significantly inhibited tumor growth and prolonged survival rates in the BALB/c syngeneic mammary carcinoma cancer models, PDL1-Vaxx and/or PD1-Vaxx targeting PD-1/PD-L1 axis as well as CTLA4-Vaxx are promising checkpoint inhibitor vaccines that can be translated to the clinic in a Phase1 clinical trial.

Translational/Clinical Research ***Faculty***

POSTER #110

1Q AMPLIFICATION AND PHF19 EXPRESSING HIGH-RISK CELLS ARE ASSOCIATED WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Multiple Myeloma is an incurable plasma cell malignancy with a poor survival rate that is usually treated with immunomodulatory drugs (iMiDs) and proteosome inhibitors (PIs). The malignant plasma cells quickly become resistant to these agents causing relapse and uncontrolled growth of resistant clones. From whole genome sequencing (WGS) and RNA sequencing (RNA-seq) studies, different high-risk translocation, copy number, mutational, and transcriptional markers can be identified. One of these markers, *PHF19*, epigenetically regulates cell cycle and other processes and is already studied using RNA-seq. In this study a massive (325,025 cells and 49 patients) single cell multiomic dataset we generate jointly quantified ATAC- and RNA-seq for each cell and matched genomic profiles for each patient. We identify an association between one plasma cell subtype with myeloma progression that we call relapsed/refractory plasma cells (RRPCs). These cells are associated with chromosome 1q alterations, *TP53* mutations, and higher expression of *PHF19*. We also identify downstream regulation of cell cycle inhibitors in these cells, possible regulation by the transcription factor (TF) *PBX1* on chromosome 1q, and determine that *PHF19* may be acting primarily through this subset of cells. Through individual patient analysis of single cell multiome data, we find evidence of PBX1 regulation of *PHF19* subclonally and differences in *TP53* mutations in these same subclones. This comprehensive analysis shows how epigenetic regulation of cell cycle driven by copy number alterations can effect intratumor heterogeneity and therapeutic resistance in relapsed multiple myeloma patients.

Translational/Clinical Research ***Faculty***

POSTER #111

ELUCIDATING THE ETIOLOGY AND IMMUNOTHERAPEUTIC TARGETS OF T CELL LARGE GRANULAR LEUKEMIA

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Background: Patients with T cell large granular leukemia (T-LGL) harbor expanded clonal CD8+T cell populations with an activated cytotoxic phenotype. The clonally expanded T cells often carry mutations in Stat3 or Stat5b genes that impede inhibitory feedback regulations. In many cases, T-LGL is accompanied by autoimmune manifestations, e.g., neutropenia, anemia, or rheumatoid arthritis. The etiology of T-LGL and the nature of autoimmune target antigens are not well understood.

Objective: We are studying the case of a patient diagnosed with T-LGL and severe neutropenia.We used the DriverMap technology ((Cellecta Inc.) for targeted RNA Sequencing of PBMC, and single-cell sorted CD3+T cells. PBMC isolated at different time points before and after chemotherapeutic treatment were subjected to differential gene expression analysis and T cell receptor (TCR) identification of the most abundant T cell clonotypes.

Results: At the time when the patient suffered severe neutropenia the gene enrichment analysis highlights significantly enriched pathways of viral interactions with cytokine and cytokine receptors, chemokine and cytokine signaling, Toll-like receptor signaling, and other activated immune and autoimmune pathways. After chemotherapeutic treatment, the activated antiviral immune pathways subside to levels found in normal PBMC. CD3+ Single-cell sorting from PBMC followed by targeted single-cell RNA sequencing revealed the presence of one highly abundant clonotype (68% clonality) and four clonotypes at moderately increased frequencies (13% clonality) at the time of severe neutropenia. The CDR3 regions of the sequenced clonal TCRs find partial matches in the VDJdb and Immunewatch DETECT databases that suggest reactivity to human cytomegalovirus (HCMV) pp65 and IE1 epitopes and one potential autoimmune epitope, BST2.

Conclusion: Our results suggest a scenario for T-LGL etiology where one or a few CD8+ cytotoxic T cell clones activated or reactivated by cytomegalovirus epitopes are immortalized by a Stat3 mutation. These highly expanded T-cell clones can be reactivated by viral surges and lead to catastrophic depletion of neutrophils that are known to disseminate HCMV and present viral epitopes. The identification of precise TCR sequences from the leukemic T cell clones will serve to design clonotype-specific immunotherapeutic agents.

Translational/Clinical Research ***Faculty***

POSTER #112

AGEING ASSOCIATED CMML DUE TO LOSS OF TET2 IS DRIVEN BY EXCESSIVE RIBOSOMAL BIOGENESIS IN A PI3K/MYC DEPENDENT MANNER.

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TET2 (Ten Eleven translocation 2) is a dioxygenase enzyme involved in 5-methylcytosine oxidation and plays a critical role in the regulation of DNA methylation. *TET2* mutations are present in 50-60% of chronic myelomonocytic leukemia (CMML) patients. Competitive repopulation studies involving *Tet2*$^{-/-}$ hematopoietic stem cells (HSCs) exhibit increased repopulating capacity, and these cells show differentiation skewing towards monocytic/granulocytic lineages, leading to leukemia with features similar to CMML. Exposure of HSCs with somatic *Tet2* mutations to acute/chronic inflammatory stimuli profoundly accelerates the development of clonal hematopoiesis (CH) and leukemia. This occurs, due in part to enhanced production of inflammatory cytokines by mutant hematopoietic stem and progenitor cells (HSPCs), which over time, in a feed-forward loop drive the survival, expansion and self-renewal of mutant HSPCs but induce apoptosis and differentiation in normal HSPCs. However, the signaling mechanism(s) downstream from these mutations in aged HSPCs, which contribute to their enhanced cellular competitiveness and production of inflammatory cytokines, are not known. PI3K signaling is essential for balanced hematopoiesis, but aberrant PI3K activity contributes to hematopoietic malignancies *via* multiple mechanisms. In adults, complete loss of PI3K activity results in reduced hematopoietic stem cell (HSC) differentiation, whereas partial loss of PI3K signaling has little impact on HSCs. Here, we show that PI3K signaling is critical for progression of *Tet2* loss induced chronic myelomonocytic leukemia (CMML) and acute myeloid leukemia (AML). Aged *Tet2*$^{-/-}$mice develop CMML and show reduced survival due to high PI3K activity and *c-Myc* levels. Remarkably, PI3K signaling blockade completely rescues *Tet2* loss induced CMML like disease by reducing inflammatory monocytes and cytokines in aged *Tet2*$^{-/-}$ mice, which is associated with a reduction in the expression of genes related to mTOR/p70S6K/eIF4 pathway as well as, oxidative phosphorylation and mitochondrial pathway. However, PI3Kloss induced myeloid skewing and reduced expression of *c-Myc* transcriptional targets in *Tet2*$^{-/-}$ HSCs, had the most dramatic impact on preleukemic HSCs, including in promoting growth and survival. PI3K/Myc inhibition inhibits proliferation and promotes apoptosis in *Tet2*$^{-/-}$ HSCs. We show that PI3K signaling is absolutely required for the development of *Tet2*$^{-/-}$ driven CMML like disease in part by regulating the levels of Myc in preleukemic HSCs.

Translational/Clinical Research ***Faculty***

POSTER #113

SHP2 PHOSPHATASE SIGNALING IS ESSENTIAL FOR DNMT3A LOSS INDUCED HEMATOPOIETIC DYSREGULATION AND MYELOID MALIGNANCY.

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DNA methyltransferase 3A (*DNMT3A*) is one of the most frequently mutated genes in patients with myeloid malignancies. Patients with *DNMT3A* mutations show hepatosplenomegaly and lymphadenopathy due to extramedullary hematopoiesis compared to patients with wildtype *DNMT3A*. Similarly, loss of *Dnmt3a* in mice results in reduced overall survival as well as hepatosplenomegaly, which is associated with myeloid cell infiltration in various tissues, myeloproliferation, and liver-specific expansion of hematopoietic stem and progenitor cells (HSC/Ps). However, the signaling pathways that induce such profound hematopoietic dysregulation are poorly understood. The non-receptor protein tyrosine phosphatase *SHP2*, encoded by *PTPN11*, is ubiquitously expressed, and regulates cell survival and proliferation and was one of the first reported oncogenic tyrosine phosphatase. However, the role of *SHP2* phosphatase in *DNMT3A* loss-induced hematopoietic dysregulation and myeloid malignancy is not known. We investigated the role of *Shp2* phosphatase in *Dnmt3a* loss-induced myeloid malignancy, and the underlying molecular mechanisms involved using genetic mouse models and pharmacological inhibitors.

Hematopoietic-specific deletion of *Dnmt3a* in mice resulted in myeloid malignancy characterized by splenomegaly, hepatomegaly with an increased percent of neutrophils, monocytes, and a decrease in lymphocyte counts. Haploinsufficiency of *Shp2* in *Dnmt3a*-null mice showed correction in peripheral blood counts with a decrease in neutrophils and increased lymphocyte counts, and improved erythroid parameters compared to *Dnmt3a*-null mice. Heterozygous loss of the *Shp2* allele in *Dnmt3a*-null mice decreased the number of myeloid progenitors, LSKs, proliferating CD48+ MPPs, CMPs, GMPs, and improved MEP cell numbers when compared to *Dnmt3a*-null mice. Similarly, spleen tissues from $Dnmt3a^{-/-}$; $Shp2^{+/-}$ mice showed a decrease in the frequency of LSKs and myeloid progenitors compared to *Dnmt3a*-null mice. Heterozygous loss of *Shp2* in *Dnmt3a*-null mice significantly decreased myeloid cell infiltration reflecting decreased liver size in $Dnmt3a^{-/-}$; $Shp2^{+/-}$ mice compared to $Dnmt3a^{-/-}$ mice. Also, haploinsufficiency of *Shp2* in *Dnmt3a* mutated mice showed a decrease in the frequency of immature myeloid cells (CD11b+, Gr1-), myeloid blasts (CD11b+, Kit+), inflammatory myeloid cells (Ly6C-hi, CD11b+), and improved B-cell development compared to *Dnmt3a*-null mice. Thorough examination for immunosuppressive cells in $Dnmt3a^{-/-}$; $Shp2^{+/-}$ mice showed a decrease in immunosuppressive Tregs, M-MDSCs, and PMN-MDSCs compared to the *Dnmt3a* knockout condition. Upon observing improved survival and decreased myeloid malignancy features in *Dnmt3a*-null mice in the setting of Shp2 haploinsufficiency, we performed SHP2 inhibitor (SHP099) treatment studies on leukemic *Dnmt3a*-null mice. SHP099-treated *Dnmt3a*-null mice exhibited a decrease in myeloid malignancy characteristics such as hepatomegaly, splenomegaly, neutrophil, monocyte counts, and improved lymphocyte counts compared to vehicle-treated *Dnmt3a*-null mice. Also, SHP099 treatment recovered LSK and myeloid progenitor numbers, and improved common lymphoid progenitor numbers (CLPs) and erythroid cell maturation when compared to vehicle-treated *Dnmt3a*-null mice. Here, we show that inhibiting Shp2 signaling using a pharmacological inhibitor or genetic depletion of *Shp-2* phosphatase reduces *Dnmt3a* loss-induced myeloid skewing, myeloid malignancy, and improves overall survival. Pharmacological inhibition of SHP2 by SHP099 is efficacious and offers a novel therapeutic approach to target *DNMT3A* mutated myeloid malignancies.

Translational/Clinical Research ***Faculty***

POSTER #114

TARGETING NLRP3 INFLAMMASOME IN JUVENILE MYELOMONOCYTIC LEUKEMIA

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Juvenile myelomonocytic leukemia (JMML) is a rare and aggressive hematological malignancy affecting young children with features of both myeloproliferation and myelodysplasia. JMML is characterized as being Ras-driven due to mutations in *NF1*, *CBL*, *KRAS*, *NRAS*, or *PTPN11*, leading to abnormal proliferation of myeloid cells. Unlike other types of blood cancers, JMML is associated with a hyperinflammatory state, which can mimic symptoms of viral infections. Traditional chemotherapy drugs are not effective in treating JMML, and the only curative treatment is allogeneic hematopoietic stem cell transplantation (HSCT). Even after transplantation, event free survival (EFS) is only 50%, with relapse being the most common cause of death. Relapse is thought to be due to the bone marrow microenvironment (BME) being altered by the hyperinflammatory state of JMML. Therefore, understanding the role of the hyperinflammatory state in JMML development and progression is important for developing more effective therapies. Studies suggest that the hyperinflammatory state in JMML is associated with hyperactive innate immune cells and hypersensitivity to GM-CSF, a cytokine that stimulates the production of myeloid cells. The hyperinflammatory state may also damage the BME, making it more favorable for the survival and expansion of leukemia cells as opposed to normal cells. In myeloid cells, inflammation can be triggered by inflammasomes, which are cytosolic multi-protein complexes that mediate host defense against microbial infection and cellular stress. Two important genes that have been implicated in this process are NLR family pyrin domain containing 3 (*NLRP3*) and prostaglandin-endoperoxide synthase 2 (*PTGS2*). NLRP3 is an inflammasome receptor and PTGS2 is an enzyme that catalyzes the synthesis of prostaglandins. How these molecules contribute to JMML associated pathologies is not known. We anticipate that the activation of the *NLRP3* inflammasome may lead to the upregulation of *PTGS2*, which in turn can promote the production of proinflammatory cytokines such as IL-1β and IL-6. This can create a positive feedback loop that sustains chronic inflammation and contributes to JMML development. Our data involving RNA-seq analysis from patients with JMML revealed elevated *NLRP3* and *PTGS2* expression compared to healthy controls. Moreover, in leukemia, high *NLRP3* and *PTGS2* expression is associated with poor overall survival as compared to patients with low expression of *NLRP3* and *PTGS2*. Consistent with the patient data, utilizing a mouse model of JMML driven by *PTPN11* mutation, which encodes the protein tyrosine phosphatase, Shp2 ($Shp2^{E76K/+}$), we have evidence to suggest that increased *Nlrp3* expression might contribute to the pathogenesis of JMML. Moreover, $Shp2^{E76K/+}$ mice displayed reduced percentages of $CD8^+$ and $CD4^+$ T-cells. Notably, both $CD4^+$ and $CD8^+$ T-cells from $Shp2^{E76K/+}$ mice had enhanced expression of terminally exhausted T (Tex) cells and regulatory T-cells (T-regs), which likely suppress T-cell-mediated antitumor responses in $Shp2^{E76K/+}$ mouse model of JMML. Importantly, loss of *Nlrp3* significantly rescued elevated number of myeloid ($Gr\text{-}1^+/CD11b^+$) cells in the PB, BM and spleens of $Shp2^{E76K/+}$ mice compared to controls. In addition, the splenomegaly observed in this model of JMML was also significantly rescued in the setting of $Shp2^{E76K/+}$;$Nlrp3^{-/-}$ mice. Moreover, the combination therapy of MCC950 (an NLRP3 antagonist) and celecoxib (a PTGS2 antagonist) rescued myeloid cell expansion, systemic inflammation and immune cell suppression and reverse the disease features in $Shp2^{E76K/+}$mice model of JMML. Given these findings, our data suggest that targeting the NLRP3/PTGS2 signaling pathway may offer a promising therapeutic strategy for JMML and leading to improved survival

outcomes for patients.

Key words: JMML, NLRP3, PTGS2, MCC950, Celecoxib.

Translational/Clinical Research ***Faculty***

POSTER #115

ALKYNYL NICOTINAMIDES WITH ANTILEUKEMIC ACTIVITY FOR TREATING POOR PROGNOSIS AML

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Alkynyl nicotinamides with antileukemic activity for treating poor prognosis AML

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Abstract

Activating mutations of *FLT3* contribute to deregulated hematopoietic stem and progenitor cell (HSC/Ps) growth and survival in patients with acute myeloid leukemia (AML) leading to poor overall survival. In 2017, Midostaurin (Rydapt), a multi-kinase inhibitor became the first targeted therapy to be approved by food and drug administration (FDA) for the treatment of AML, followed by Gilteritinib (Xospata), a FLT3 and AXL1 specific inhibitor in 2018. Additional experimental drugs specific for mutant FLT3 in various stages of clinical trials including Quizartinib and Crenolanib have also been described, although they are known to develop both intrinsic and acquired resistance in response to FLT3 targeted therapy. More recently, the emergence of RAS mutations has been reported in AML patients treated with Gilteritinib. Thus, there is a critical unmet need to identify and develop potent and selective inhibitor(s) for mutant FLT3 to provide additional therapeutic options for treating AML patients with these mutations. Despite the high prevalence of *FLT3* mutations and their clinical significance in AML, there are few targeted therapeutic options available. To this end, we have recently identified a novel class of nicotinamide-based *FLT3* inhibitors (HSN608 and HSN748) that specifically target *FLT3* mutations at sub-nanomolar concentration and are potently effective against drug-resistant secondary mutations of *FLT3*. These compounds show antileukemic activity against $FLT3^{ITD}$ in drug-resistant AML, relapsed/refractory AML, and in AML bearing a combination of epigenetic mutation, *TET2* along with $FLT3^{ITD}$. We demonstrate that HSN748 outperforms the FDA-approved *FLT3* inhibitor Gilteritinib in terms of inhibitory activity against $FLT3^{ITD}$ *in vivo.*

Translational/Clinical Research Faculty

POSTER #116

MUTANT RAS-DRIVEN SECRETOME CAUSES SKELETAL MUSCLE DEFECTS IN BREAST CANCER

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Cancer-induced skeletal muscle defects differ in severity between individuals with the same cancer type. Cancer subtype-specific genomic aberrations are suggested to mediate these differences, but there are limited efforts to link specific genomic aberrations with skeletal muscle defects. We utilized three different breast cancer patient-derived xenograft (PDX) models to correlate cancer subtype with skeletal muscle defects. PDXs were derived from brain metastasis of triple negative breast cancer (TNBC), Estrogen Receptor-positive/Progesterone Receptor-positive (ER+/PR+) primary breast cancer with BRCA2-mutation, and pleural effusion from an ER+/PR- breast cancer patient. While impaired skeletal muscle function as measured through rotarod performance and reduced levels of circulating and/or skeletal muscle *miR-486* were common across all three PDXs, only TNBC-derived PDX activated phospho-p38 in skeletal muscle. To further extend these results, we generated transformed variants of human primary breast epithelial cells from healthy donors using $HRAS^{G12V}$ or $PIK3CA^{H1047R}$ mutant. Mutations in *RAS* oncogene or its modulators are found in ~37% of metastatic breast cancers, which is often associated with skeletal muscle defects. Although cells transformed with both oncogenes generated adenocarcinomas in NSG mice, only $HRAS^{G12V}$-derived tumors caused skeletal muscle defects affecting rotarod performance, skeletal muscle contraction force, and *miR-486, Pax7*, pAKT, and *p53* levels in skeletal muscle. Circulating levels of the chemokine CXCL1 were elevated only in animals with tumors from $HRAS^{G12V}$ mutation. Since RAS pathway aberrations are found in 19% of cancers, evaluating skeletal muscle defects in the context of genomic aberrations in cancers, particularly RAS pathway mutations, may accelerate development of therapeutic modalities to overcome cancer-induced systemic effects.

Translational/Clinical Research ***Faculty***

POSTER #117

NATIONAL UTILIZATION OF COMPLETION LYMPHADENECTOMY AFTER MULTIPLE POSITIVE SENTINEL LYMPH NODES IN PATIENTS WITH MELANOMA

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INTRODUCTION: MSLT-II/DeCOG trial results have made nodal observation acceptable in lieu of completion lymph node dissection (CLND) in patients with a positive sentinel lymph node (SLN). However, 70% of the patients in MSLT-II and 90% in DCOG had 1 positive SLN, and national use of CLND in patients with >=2 positive SLN is poorly characterized. The objectives of this study were (1)to assess national trends of CLND use in patients with >=2 positive SLN vs 1 positive SLN and (2)to examine predictors of CLND use.

METHODS: From the NCDB (2013-2021), stage I-III melanoma patients were identified who underwent a SLNB. Patients were stratified as having >=2 positive SLNs vs. 1 positive SLN. Factors associated with CLND were assessed by multivariable logistic regression.

RESULTS: Of 139,410 patients who underwent SLN biopsy, 5,070(3.6%) had >=2 positive SLNs. CLND rates for >=2 positive SLNs decreased by 61% (75% to 14%, P<0.001), while CLND rates for 1 positive SLN decreased 41% (50% to 9%, P<0.001). Between 2018-2021, patients with >=2 positive SLNs were more likely to undergo CLND if they had head/neck melanoma (OR=1.82 95%CI 1.13-2.92), and if ulcerated (OR=1.47 95%CI 1.06-2.04). Patients were less likely to undergo CLND if they had lower extremity melanoma (i.e. inguinal node dissection; OR=0.47 95%CI 0.28-0.77).

CONCLUSION: While patients with >=2 positive nodes were infrequent in MSLT-II/DeCOG, results of the trial have been extended to this population. Long-term outcomes should be examined to assess the oncologic safety of this practice.

Translational/Clinical Research ***General Surgery Resident, Masters in Clinical Research***

POSTER #118

PRECLINICAL MODELING OF CDK4/6 INHIBITORS FOR LATE-STAGE NF1 TUMORS

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Purpose: The leading cause of premature death for patients with Neurofibromatosis type 1 (NF1) is the development of malignant peripheral nerve sheath tumors (MPNSTs) which render current medical treatment strategies largely ineffective, prompting an investigation of rational targeted therapies opposing the chronic cell cycle deregulation that promotes this malignant transformation.

Methods: Molecular and phenotypic responses to the targeted CDK4/6 inhibitors abemaciclib and palbociclib were evaluated in three human MPNST cell line models (NF1 and CDKN2A null). Multiplexed kinase inhibitor bead (MIB) affinity chromatography coupled with mass spectrometry (MIB/MS) was used to analyze changes in kinase activity and expression following CDK4/6 inhibitor treatment, compared to DMSO control. RB1 knockout clones were generated using CRISPR/Cas9 to evaluate the dependence of CDK4/6 inhibition on RB, on account of clinical studies identifying RB1 loss as a potential mechanism for acquired resistance.

Results: MPNST cell lines demonstrated sensitivity to single agent treatment by CDK4/6 inhibition, with abemaciclib providing a greater reduction in cell viability than palbociclib. Both inhibitors elicited a diverse change in kinome profile compared to control, primarily exhibiting a reduced MIB binding of kinases that reflect a cell cycle arrest phenotype. Previously reported off-targets exclusive to abemaciclib were also identified as having reduced binding during MIB/MS. RB1 knockout MPNST clones exhibited resistance to palbociclib but remained sensitive to abemaciclib in long-term exposure studies. Abemaciclib treatment also outperformed single agent inhibition of its non-CDK targets in both RB1 proficient and knockout cells.

Conclusions: CDK4/6 inhibition shows promise as a targeted therapy against late-stage NF1 tumors with dysregulated cell cycle signaling. Single agent treatment with abemaciclib and palbociclib suppressed growth of MPNST cell lines, but only abemaciclib remained effective upon knockout of RB1. Lack of resistance in these clones, along with a unique kinase signature revealed by MIB/MS analysis, suggests the importance of off-target binding of abemaciclib in its effect on cell proliferation. Further characterization of abemaciclib treatment is therefore warranted, including the potential for synergistic combination therapies through other kinase targets.

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

POSTER #119

NON-INVASIVE MRI EVALUATION OF TUMOR-ASSOCIATED MACROPHAGES AFTER RADIOTHERAPY IN A DUAL TUMOR MOUSE MODEL

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Purpose: Tumor-associated macrophages (TAMs) lead to an immunosuppressive tumor microenvironment and treatment resistance in metastatic breast cancer. Evaluation of TAMs could help predict patients' prognosis or treatment outcome. Ferumoxytol has been used as a magnetic resonance imaging (MRI) probe for non-invasive evaluation of TAMs. However, it is unknown whether ferumoxytol MRI reliably measures TAMs after radiotherapy. This study addresses this by evaluating the capability of ferumoxytol MRI for measuring TAMs after radiotherapy in a dual tumor mouse mammary cancer model.

Methods: Fourteen mice were subcutaneously implanted with 4T1 primary orthotopic (mammary fat pad) and secondary tumor (flank) in BALB/c mice. Mice were separated into two groups: radiation and no radiation (n=7 mice per group; 28 tumors). The primary tumor of the mice in radiation group received radiation (8-Gy×3) on days 15-17 post tumor implantation. At 23 days post tumor implantation, MRI R2* maps were acquired pre-injection and 24-h post-ferumoxytol injection. After MRI, both tumors were harvested and analyzed for anti-tumoral (M1, $CD206^{-}CD80^{+}$) and pro-tumoral (M2, $CD206^{+}CD80^{-}$) macrophages by flow cytometry.

Results: After ferumoxytol injection, the median R2* change of the primary tumor and the secondary tumor in the no radiation group was 36.8% (p=0.02) and 24.2% (p=0.02), respectively. In the radiation group median R2* changes of the primary tumor and the secondary tumor were 41.3% (p=0.02) and 39.9% (p=0.02), respectively. Positive Pearson correlations were found in the no radiation group between M1 macrophages and mean R2* changes (r=0.79; p=0.007) but this correlation was not found in the radiation group.

Conclusions: In this mouse model, significant ferumoxytol tumor retention was observed in all treatment groups. A positive correlation between M1 macrophages and mean R2* changes was found in untreated tumors but not in tumors treated with radiation, suggesting ferumoxytol MRI may not be a reliable marker of macrophages after radiotherapy.

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

POSTER #120

3D PRINTING TECHNOLOGY IN SPATIALLY FRACTIONATED RADIATION THERAPY COLLIMATOR DESIGN FOR PRECLINICAL APPLICATIONS: A NOVEL, CUSTOMIZABLE, AND COST-EFFECTIVE APPROACH

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Objective:

Emerging evidence suggests that spatially fractionated radiation therapy (SFRT) may provide similar efficacy as conventional radiotherapy but with fewer toxic complications, introducing an invaluable treatment option for previously untreatable bulky tumors. SFRT utilizes non-uniform dose distributions characterized by high "peak" doses separated by low dose "valleys". However, the underlying working mechanisms of SFRT have not been established, making clinical translation challenging. This project describes a simple, readily-customizable, yet cost-effective method for fabricating collimators that can be utilized to investigate SFRT mechanisms in preclinical experiments.

Methods:

Collimator prototypes for delivering SFRT were designed in Fusion 360 and 3D-printed using a Formlabs resin printer. The design consists of a resin shell with resin pillars acting as 'holes' to deliver peak doses. The shell is then filled with 1-mm diameter tungsten ball bearings as the shielding material to create valley doses. For dosimetric characterization, measurements were acquired per TG-61 guidelines utilizing a NIST-traceable cylindrical farmer chamber in addition to a phantom consisting of alternating layers of 5-mm bolus and calibrated Gafchromic film. SFRT collimator characteristics were obtained in an XRAD 320 small-animal cabinet irradiator.

Results:

An SFRT collimator prototype optimized for mouse tumors was fabricated, consisting of sixteen 1.6-mm diameter resin pillars. The tungsten-filled 3D-printed shell yielded a 4.5-mm effective thickness. Dosimetric characterization results at 10-mm depth include a 3.10-Gy mean peak dose, 0.32-Gy mean valley dose, and a mean peak-to-valley dose ratio of 9.80. Physical characteristics of the collimator include: 1.7-mm peak width at half-maximum, 1.2-mm valley width, and a 2.9-mm peak-to-peak distance.

Conclusion:

A novel collimator design utilizing 3D printing technology and commercially available tungsten ball bearings has been fabricated and characterized for our preclinical treatment system. This collimator design technique is user-friendly, allows for easy implementation, and is readily customizable for preclinical applications to investigate SFRT mechanisms.

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

POSTER #121

TOBACCO USE, KNOWLEDGE OF HARMS, AND CESSATION SUPPORT AMONG PATIENTS WITH NON-TOBACCO-RELATED CANCERS

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Introduction

Cigarette smoking is responsible for approximately 30% of cancer deaths in the United States. Even among patients with non-tobacco-related cancers such as breast, colon, and prostate cancer, continued cigarette smoking among patients with cancer leads to numerous adverse health outcomes. Continued smoking is associated with poorer response to cancer treatment, increased risk for treatment-related toxicities, and shorter overall survival. While some patients with smoking-related cancers, such as lung cancer, make efforts to quit smoking at the time of diagnosis, patients with other forms of cancer might not understand the negative effects of continued smoking. In this study, we assessed patient knowledge of the harms of continued smoking, previous cessation attempts, and cessation support.

Methods

We conducted a multicenter cross-sectional survey study among 124 adults with a diagnosis of non-tobacco-related types of cancer at an NCI-designated cancer center and an urban safety-net medical center. Participants were asked about their current smoking behaviors, beliefs about the harms of continued smoking, attempts to quit and resources used, and cessation support. We performed a standard descriptive statistical analysis on patients who smoked within the previous 30 days to summarize and present the collected data.

Results

79 out of the 124 participants were female (63%), 32 (25%) were Black, and 88 (70%) were White. Most participants had either breast (62, 50%), colorectal (30, 24%), or prostate cancer (24, 19%); other less common malignancies included testicular, sarcoma, melanoma, esophageal, and lymphoma. 62 (50%) of the participants smoked one pack or more per day. Most patients (109, 87%) agreed or strongly agreed that continued smoking may shorten life expectancy, and 97 (78%) agreed or strongly agreed that continued smoking increased the risk of getting a different type of cancer. Only 57 (46%) patients agreed or strongly agreed that continued smoking may cause more side effects from cancer treatment, and only 57 (46%) agreed or strongly agreed that ongoing smoking may affect treatment response. Most patients (101, 81%) had tried to quit smoking for good, with a median of 4 quit attempts. 110 participants (88%) reported that their physicians had advised them to quit smoking; however, only 52 (41%) were recommended or prescribed a medication to help them quit, and only 52 (41%) received follow-up on cessation attempts.

Conclusion

Among 124 patients with non-smoking-related malignancies who currently smoke, there was incomplete knowledge of the harms of continued smoking. Oncologists frequently advise patients to quit; however, they less frequently prescribe medication or follow up on cessation efforts. Interventions are needed to educate patients with cancer about the harms of continued smoking and to provide further cessation support.

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

POSTER #122

ENHANCING NUCLEIC ACID HYBRIDIZATION TECHNOLOGY FOR FAST AND SELECTIVE MICRORNA DETECTION IN BLOOD PLASMA UTILIZING PLASMONIC NANOSENSORS

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MicroRNAs are short non-coding RNAs consisting of 18-25 nucleotides and have been implicated as key biomarkers for various diseases such as diabetes, cancers, heart disease, autoimmune, and possibly even Alzheimer's. Therefore, sensitive and selective detection/quantification of microRNAs are important to for early disease diagnostics. MicroRNA detection techniques that are traditionally used in clinics such as northern blotting, microarray analysis, and polymerase chain reaction show several disadvantages from long hybridization time, possible loss of low concentration microRNAs during time consuming and extensive sample processing steps, and signal amplification that prevent their use in clinical point-of-care. Other laboratory-based microRNA detection/quantification methods such as electrochemical, fluorescence, surface plasmon resonance, and surface-enhanced Raman scattering heavily rely on the receptor (i.e.,-single-stranded DNA, or aptamers)/microRNA duplex formation. However, microRNA hybridization process is restricted by mass-transport limited diffusion process in which the diffusion constant of microRNAs is 10^{-6} to 10^{-7} cm^2 s^{-1} in solution which limits the microRNA detection time to several hours for microRNAs to reach an equilibrium in binding to the receptors. Thus, there is a need to fundamentally explore different approaches to speed up the receptor/microRNA duplex formation so that the translational aspect of the technology can be fully realized by implementing it in point-of-care diagnostics. Herein, we examine how the receptor/microRNA interaction can be modulated to enhance the kinetics of microRNA hybridization process and result in the development of a fast and real-time nucleic acid detection technology. We report that small molecule intercalators enhance the -ssDNA/microRNA binding kinetics in low ionic strength PBS buffer. We utilized the localized surface plasmon resonance (LSPR) properties of gold triangular nanoprisms to characterize ssDNA/microRNA binding kinetics in solid-state in the form of nanoplasmonic sensor construct. We experimentally observe that the presence of acridine orange intercalator promotes a faster association between the -ssDNA/miRNA duplexes. Furthermore, our experimental result is supported by the molecular dynamic simulation results on model -ssDNA/microRNA duplex systems calculating the free energy of the system as a function of the distance of intercalators from the -ssDNA/microRNA duplex. As a proof-of-concept, we were able to detect and quantify micrRNA-10b and -21 in femtomolar/microliter concentrations directly in plasma from a cohort of 300 human plasma samples including 100 pancreatic cancer (PDAC), 100 intraductal papillary mucinous neoplasms (IPMN), and 100 healthy control (HC) samples with 2 h of sample incubation time and a total 5 h assay time in a high-throughput 384 well-plate platform. Our detection scheme not only successfully differentiates between different pancreatic diseases (i.e., PDAC vs. IPMN), but also differentiate different stages of pancreatic cancer with extremely high selectivity. Importantly, our nanoplasmonic-based microRNA detection technology shows outstanding correlation with the digital droplet-PCR validation method. We believe our work on label- and amplification-free nucleic acid detection methods is capable of providing fundamental insights of modulating the nucleic hybridization process, which can potentially be used as real time point-of-care diagnostic tool by analyzing human biofluids for cancers and other diseases.

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

POSTER #123

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

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Breast cancer is the most commonly diagnosed cancer in women and is the second leading cause of cancer-related deaths in women. Approximately 20-30% of patients will develop metastases and metastasis is responsible for greater than 90% of breast cancer deaths. Metastasis is a complex process in which the cells combat many forces to survive and spread to different areas of the body. We have previously shown that heat shock factor 1 (HSF1) promoted epithelial-to-mesenchymal transition (EMT) and the breast cancer stem-like population, potentially linking HSF1 to metastasis. Utilizing an HSF1 gene expression signature that assesses HSF1 transcriptional activity, we further found that patients with high HSF1 activity have significantly worse metastasis-free survival. HSF1 is the master regulator of the heat shock response wherein it upregulates chaperone proteins under stress conditions. Because the process of metastatic colonization is known to involve the stem cell population and incur external stressors, we hypothesized that HSF1 may function in metastatic colonization. To test this, we subjected human breast cancer MDA- MB-231 cells with or without HSF1 knockdown to intracardiac injections in nude mice. Mice receiving cells with knockdown of HSF1 had a significantly reduced metastatic burden, indicating HSF1 is necessary for the completion of metastasis and colonization. Consistent with these findings, bone metastatic tumor specimens from patients show increased HSF1 activation compared to their matched primary breast tumors. We observed increased protein amyloid aggregates that correlate with an increase in HSF1 activity during anchorage-dependent colony formation, suggesting that colonization induces aggregation leading to HSF1 activation that promotes a cell survival response. Overall, my results indicate that HSF1 activity increased during clonogenic formation and HSF1 is necessary for colonization. Going forward, I want to understand what controls HSF1 activation during metastasis, which I hypothesize that protein aggregation is increased leading to HSF1 activation.

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

POSTER #124

CREATION AND CHARACTERIZATION OF A NOVEL ADENOMYOSIS EPITHELIAL CELL LINES

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Adenomyosis occurs when endometrial-like tissue is found within the wall of the uterine myometrium. Typically, it is a benign disease with symptoms of abnormal uterine bleeding that results in a hysterectomy. However, adenomyosis is frequently found in hysterectomy specimens containing endometrial cancer. The study of adenomyosis as a benign disease and its relationship to endometrial cancer is limited by a lack of in vitro models. Here, we describe the first telomerase-transformed epithelial cell line derived from human adenomyosis.

With IRB approval, the IUSCCC Biospecimens Collection and Banking Core (BC^2) procured endometrial tissue from a hysterectomy. Final surgical pathology revealed focal adenomyosis of the uterus. Sample processing included enzymatic digestion, isolation, and separation of epithelial and stromal cells via the selective adhesion method, and fluorescence-activated cell sorting using an 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 EpCAM+ cells rapidly grew and were easily immortalized with the human telomerase reverse transcriptase gene (hTERT). Testing and 16-marker short tandem repeat (STR) profiling (IDEXX BioAnalytics) confirmed the cells as human, not contaminated with mycoplasm, and to be not a known cell line contaminant. Basic immunofluorescent characterization of the immortalized cells confirmed that the line, designated as tEEC21, was positive for the epithelial markers cytokeratin-5 (cytoplasmic) and N-cadherin (membrane and cell junctions) and negative for the stromal marker CD10 (cytoplasmic). To further characterize the cells, nuclear immunostaining showed positive staining for paired box 8 (PAX8) which stains positive in endometrial, cervical, and fallopian tube epithelium. Consistent with endometrial-like epithelium, nuclear staining was highly positive for HNF1B and ARID1A. Interestingly, cytogenetic analysis on G-banded metaphase spreads indicated polyploidy with multiple chromosomal rearrangements. Consistent with a non-benign, epithelial cell type, tEEC21 was positive for cytoplasmic NAPSINA, a marker for ovarian clear cell or renal cell carcinoma. Future studies are focused on steroid hormone response. The establishment and delineation of this new cell line will serve as a research resource for adenomyosis and adenomyosis-associated gynecologic cancers.

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

POSTER #125

TESTING PRECISION GENOMICS-GUIDED THERAPY IN A MODEL OF ANAPLASTIC PLEOMORPHIC XANTHOASTROCYTOMA: DUAL INHIBITION OF CDK4/6 AND MEK

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Pediatric brain tumors are the second most common and deadliest form of childhood cancer. Pediatric high-grade gliomas (pHGG) specifically have no effective medical treatments. Additionally, radiation and surgery do not show a long-term benefit for pHGG, resulting in typical survival of fewer than 18 months. Development of effective therapeutics against pHGG has been a challenge, due in large part to difficulty creating accurate models for these tumors. Hyperactivating mutations in the MAPK and CDK4/6 cell cycle pathways are common in pediatric gliomas and are targetable drivers of their tumorigenesis and malignant transformation. We have established a novel patient-derived xenograft (PDX) model, PDX RHT128, from a pediatric patient with a bona fide anaplastic pleomorphic xanthoastrocytoma (APXA), a type of high-grade glioma. Clinical precision genomics analysis of the tumor revealed a novel BRAF chromosomal rearrangement and deletion of CDKN2A/B, which are implicated in MAPK and CDK4/6 pathway hyperactivation, respectively. Based on this molecular signature, the patient was treated with MEK inhibitor trametinib as a monotherapy and, following progression of disease, with CDK4/6 inhibitor ribociclib. However, the tumor continued to progress. Recurrence in response to single-agent targeted treatments is a concern, as crosstalk between hyperactive CDK4/6 and MAPK pathways can overcome monotherapy to promote tumor growth. Based on this rationale, we aim to simultaneously target the CDK4/6 and MAPK pathways in our characterized APXA PDX model, which exhibits molecular fidelity to the patient's tumor, including the presence of a BRAF fusion and CDKN2A/B loss. In this study, we are investigating tumor response at the cellular and molecular levels with an in-depth analysis of CDK4/6 inhibitors. We have completed a single-agent efficacy assessment of two FDA-approved CDK4/6 inhibitors in a subcutaneous model of APXA, showing significant dose-dependent reduction in RHT128 tumor volume after treatment with abemaciclib ($p<0.05$) and palbociclib ($p<0.01$). Analysis of the global kinome in CDK4/6 inhibitor-treated PDX tissues compared to vehicle treatment using multiplexed-inhibitor bead chromatography–mass spectrometry (MIB/MS) demonstrated effective inhibition of CDK4/6. Moreover, this analysis revealed dose-dependent alterations in the MAPK and PI3K pathways and modulation of critical neurotransmitter pathways in treated tissues. Additionally, results from a separate study in our laboratory demonstrated that the blood-brain barrier-permeable MEK inhibitor mirdametinib also reduced tumor volume in this PDX model. These findings highlight the potential of combining these two strategies for treating APXA. Future studies will explore efficacy and underlying mechanisms of these MEK and CDK4/6 inhibitors in our expanded APXA

models, which include BRAF wild-type and BRAF V600E variants. The pervasiveness of alterations to the MAPK and CDK4/6 pathways in pediatric gliomas present this therapeutic strategy as a promising approach for further study and development in a broader range of these deadly tumors.

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

POSTER #126

INVESTIGATING DRIVERS OF RECURRENCE IN PEDIATRIC PILOCYTIC ASTROCYTOMA PATIENT-DERIVED CELL LINES

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Pilocytic astrocytoma (PA) is the most common brain tumor in children and considered benign, as surgical resection is often curative. However, recurrence of PA has been reported even after gross total resection, leading to increased morbidity and lower overall survival compared to less aggressive PA. Recurrence in PA remains understudied, and little is known about molecular differences between recurrent and non-recurrent PA. Alterations to the MAPK pathway, especially through BRAF mutations, are common in PA and thought to be sufficient for development of pediatric low-grade gliomas (pLGG) such as PA. Additionally, deletion of the tumor suppressor CDKN2A/B has been associated with malignant transformation and aggressive phenotypes in gliomas, including pLGG. Experimental investigation of PA has been limited by challenges establishing high-fidelity pLGG cell cultures from patients, largely due to slow growth and senescence of cell cultures. Thus, studies of recurrent PA have focused on identifying associations of molecular characteristics with aggressive or recurrent phenotypes in bulk tumor tissue after resection, without experimental investigation of the ability of these alterations to drive tumor recurrence. Enrichment of stem cell populations in patient-derived cell cultures has previously been successful in improving the fidelity and stability of pLGG cell cultures. Isolation of cancer stem-like cells allows for the study of molecular drivers of tumor recurrence in this cell population in which these driving alterations are proposed to occur. We have established cell lines from 4 pediatric PA patient tumor tissues from Riley Hospital and have grown these cells in neural stem cell media to enrich for stem-like populations. We aim to characterize stem-like features and the mutational landscape of these cell lines with the objective of genetically manipulating proposed drivers of recurrence in these cell lines through CRISPR-Cas9 targeting. Within our panel of 4 PA cell lines, we have found differences in growth rates in culture despite low-grade diagnoses of these tumors. Further, clinical molecular testing identified varied molecular backgrounds among these tumors, especially regarding BRAF status. These findings suggest differences in the underlying molecular drivers of these tumors despite PA diagnosis. Examination of neural stem-like markers by RT-qPCR and immunocytochemical staining has shown expression of markers such as nestin and SOX2 in these lines. Future studies will further characterize features of stemness in these lines and specifically study CDKN2A/B loss as a potential driver of aggression and recurrence in these PA cells. We will employ CRISPR-Cas9 technology to induce genetic knockout of CDKN2A/B in these lines and investigate phenotypes of aggression and recurrence through assays *in vitro* and *in vivo*. These studies have the potential to experimentally identify molecular risk factors for recurrence in PA which could impact molecular diagnostics and provide rationale for therapeutic targeting of these molecular alterations to prevent recurrence.

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

POSTER #127

EXPLORING THE ROLE OF KMT5B IN CANCER

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Lysine methylation is a widespread and important post-translational modification (PTM) that frequently occurs on histone and non-histone proteins in the cellular landscape. Lysine methylation is written by lysine methyltransferases (KTMs), which are frequently dysregulated in many diseases, like in many human cancers. It is also well understood that due to this dysregulation, the patterns of histone lysine methylation are altered in cancer. Lysine N-Methyl Transferase, or KMT5B (also known as SUV420H1) is a lysine methyltransferase responsible for producing histone modifications H4K20me2 and H4K20me3, modifications usually associated with transcriptional repression and DNA repair. KMT5B is considered a high impact risk gene implicated in many neurodevelopmental disorders like autism spectrum disorder (ASD) and other motor disorders. Despite its heavy involvement in neuronal development, recent studies have also shown the role of KMT5B in various cancers, including glioblastoma (PMID34447744), diffuse gliomas and hepatocellular carcinoma (PMC10859612), which can occur due to mutations altering the function of the KMT, hence altering methylation patterns and signaling pathways. Many KMTs methylate histone and non-histone proteins, and have illustrated substrates connected to them as well, but it is yet to be known if KMT5B has any non-histone substrates. Our work aims to use a functional proteomics platform to characterize the substrate specificity of KMT5B, and to help identify its non-histone substrates. This will further solidify our understanding of the signaling pathways impacted due to lysine methylation in the cellular environment, and functional modifications occurring, if any. This knowledge could support a search for identifying therapeutic inhibitors which can best target KMT5B to treat the symptoms caused in cancer.

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

POSTER #128

A NOVEL INDUCED PLURIPOTENT STEM CELL MODEL OF SCHWANN CELL DIFFERENTIATION REVEALS NF2-RELATED GENE REGULATORY NETWORKS OF THE EXTRACELLULAR MATRIX

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Schwann cells play a critical role in the formation and maintenance of the peripheral nervous system, and their dysfunction is associated with various neurological and neoplastic disorders, including NF2-related schwannomatosis. To advance our understanding of Schwann cell biology in health and disease, we have established an innovative model using human-induced pluripotent stem cells (hiPSCs). Through a comprehensive analysis combining transcriptomics, immunofluorescence, and morphological assessments, we characterized the differentiation of hiPSC-derived Schwann cell precursors (SPCs) into fully differentiated Schwann-like cells (SLCs), spanning different developmental stages. To validate our findings, we conducted integrated analyses across multiple datasets, including those from human amniotic mesenchymal stem cell (hAMSCs) derived SLCs and in vivo mouse models. Our state-of-the-art hiPSC model revealed both commonalities and unique features in Schwann cell gene expression signatures, shedding light on species-specific aspects of Schwann cell biology. Moreover, we identified dynamic gene co-expression modules associated with ear and neural development, cell fate determination, NF2 gene function, and extracellular matrix organization during hiPSC to SLCs differentiation. By cross-referencing results from various analyses, we identified potential novel genes linked to NF2, such as ANXA1, CDH6, COL1A1, COL8A1, MFAP5, IGFBP5, FGF1, AHNAK, CDKN2B, LOX, CAV1, and CAV2 for further investigation. Overall, our hiPSC model offers a versatile platform for investigating Schwann cell development in the context of human diseases, providing valuable insights into the underlying mechanisms and potential therapeutic targets.

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

POSTER #129

MULTIPLE MOLECULE Λ-DYNAMICS: PROBING ANTICANCER DRUG RESISTANCE WITH CONCURRENT PROTEIN & LIGAND PERTURBATIONS

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Computational tools are used to accelerate the identification of lead compounds in a drug discovery virtual screen and are now more heavily utilized in structure-based drug design and lead optimization. Alchemical free energy (AFE) calculations are frequently utilized to predict relative binding affinities for protein-ligand complexes, however, issues of drug resistance have yet to be widely explored with these methods. My research aims to investigate and expand the use of AFE calculations to predict the loss of binding due to missense mutations that cause clinical drug resistance. λ-dynamics is a powerful AFE tool. In contrast to previous AFE calculations that require many simulations to study a single structural modification, λ-dynamics can test many modifications within a single simulation, increasing in silico efficiency. My work seeks to advance the application of λ-dynamics to structure-based drug design involving drug resistance by using it to predict changes in ligand binding potency due to ligand functional group modifications coupled with protein side chain mutations performed simultaneously in a method called Multiple Molecule λ-Dynamics (MMλD). Our initial investigation of MMλD focuses on a ligand series designed to bind the ABL kinase, a key target in chronic myelogenous leukemia (CML) and well known for clinical drug resistance. In comparisons of predicted binding free energies from MMλD to traditional single-molecule λ-dynamics, across several independent trials including wildtype and mutant data, we observed an average Pearson R of > 0.9 and a mean unsigned error of < 0.5 kcal/mol between single-molecule and multiple molecule λ dynamics simulations. These highly accurate predictions between methods suggest MMλD can model perturbations on multiple molecules without loss in accuracy and be applied successfully to drug discovery involving drug resistance. Hence, we expect MMλD to be generalizable to many targets and diseases, helping guide future drug discovery efforts.

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

POSTER #130

TARGETING CDK4/6 AND BET PROTEINS FOR THE TREATMENT OF RB+ AND RB- OSTEOSARCOMA

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Osteosarcoma (OS) is the most common malignant bone cancer characterized by heterogeneity and genomic instability. Treatment and survival of patients have not improved in several decades, making it urgent to develop safe and effective therapies. Dysregulation of cell cycle regulators *CDKN2A*, cyclins, and CDK4/6 represent high-risk signatures in aggressive OS and can be targeted via CDK4/6 inhibitors (CDK4/6i). However, monotherapy often fails due to resistance from speculated RB loss as well as hyperactivation of compensatory signaling pathways in RB proficient cancers. In addition, >70% of OS patients have RB loss which is viewed as a biomarker of therapeutic response that negates the use of CDK4/6i. However, more research is needed in RB-deficient preclinical models to clearly define and validate the utility of RB as a biomarker for patient stratification. Based on our screening data, we hypothesize that combining CDK4/6i with bromodomain and extraterminal domain inhibitors (BETi) will potentiate anti-tumorigenic effects regardless of RB status in OS. BETi creates imbalances between transcription and replication which worsens oncogenic stress. In OS cell lines, combination index and Bliss analysis indicated additive-to-synergistic growth inhibition using CDK4/6i (abemaciclib or palbociclib) and BETi (AZD5153). Dual therapy also resulted in increased apoptosis at clinically relevant concentrations in an OS xenoline (TT2) compared to monotherapy. To determine potential for emergence of therapeutic resistance, an in-vitro longitudinal growth delay assay was established. While single agent therapies resulted in transient decreases in cell numbers over time, combination CDK4/6i+BETi resulted in cell death ($p<0.05$). To determine the effect of RB loss on therapeutic response to CDK4/6i, CRISPR mediated RB-knockout (KO) clones were developed in 143B human and K7M2 murine OS cell lines. RB KO cells exhibited sensitivity to CDK4/6i irrespective of RB status. However, levels of CDK4/6i required for 50% growth inhibition in RB KO were 1-3-fold higher using palbociclib, with negligible differences observed with abemaciclib in RB KO vs WT clones. To determine if therapeutic response is achievable in-vivo, a single-agent dosing study was conducted using palbociclib (40mg/kg, SID) and AZD5153 (1mg/kg, BID) in patient-derived xenograft (PDX) model derived from a metastatic lesion in the pelvis (TT2 PDX) harboring a CDK4/6 hyperactivation signature and monoallelic RB loss. Tumor growth reduction and survival was significantly higher in monotherapy groups compared to vehicle. However, one week after dosing was stopped, survival rates based on tumor volume endpoint were 40% and 100% for palbociclib and AZD5153 respectively. In-vivo efficacy of dual CDK46i+BETi is underway. This data provides rationale for further study of novel therapeutic options which may expand the clinical utility of CDK4/6i regardless of RB status.

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

POSTER #131

TARGETING PLATINUM-INDUCED OVARIAN CANCER STEM CELL PLASTICITY WITH VITAMIN D

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Most women diagnosed with late-stage high grade serous ovarian cancer (HGSOC) develop recurrent, platinum-resistant tumors. Ovarian cancer stem cells (OCSCs) are hypothesized to contribute to the emergence of these resistant tumors. CSCs have been postulated to reside in a plastic state, which allows for the conversion of non-CSC to CSC. This process of dedifferentiation continues during tumor development and environmental stressors, such as chemotherapeutic agents like platinum can induce CSC plasticity. We have previously demonstrated that platinum treatment enriched for OCSCs. However, whether platinum converts non-OCSCs into OCSCs to enrich OCSCs in tumors remains unclear, and the underlying mechanism remains incompletely understood. To examine OCSC plasticity, aldehyde dehydrogenase (ALDH; functional marker) and fluorescence activated cell sorting were used to isolate OCSCs (ALDH+) and non-OCSCs (ALDH-) from HGSOC cell lines, OVCAR5 and OVCAR3. To determine the stability of the non-OCSC phenotype, ALDH- cells were cultured for 3 and 5 days and ALDH activity was measured using flow cytometry. At both timepoints examined, ALDH- cells remained ALDH-, with approximately less than 1% being ALDH+ ($p<0.05$). To determine if platinum can induce conversion of non-OCSC to OCSC, ALDH- cells were treated with cisplatin (IC50, OVCAR5: 12μM, OVCAR3: 15μM,16h), and the percent of ALDH+ cells was measured using flow cytometry. Treatment of ALDH- cells with cisplatin increased ($p<0.05$) the percentage of ALDH+ cells by approximately 4% and 10% in OVCAR5 and OVCAR3 cells, respectively, and the converted cells displayed increased expression ($p<0.05$) of stemness genes *BMI1, NANOG, OCT4,* and *SOX2*. Furthermore, spheroid formation by converted cells was increased ($p<0.05$) compared to parental ALDH+ and whole cell populations treated with cisplatin, suggesting that platinum induced the observed differences in the stemness phenotype were due to platinum exposure. With the overall goal of inhibiting platinum-induced OCSC conversion by targeting key genes and pathways to inhibit platinum-induced OCSC conversion, RNA-sequencing was performed using converted OCSCs. Activated pathways in converted OCSCs included protein kinase signaling, inhibition of matrix metalloproteases, SPINK1 pathway and VDR/RXR activation. Given the known role of vitamin D receptor (VDR) signaling in cell differentiation, the VDR pathway was investigated, along with its binding partner implicated in stemness, retinoid x receptor (RXR). To determine the effect of vitamin D on stemness, cells were treated with vitamin D (OVCAR5: 34nM, OVCAR3: 56nM, 24h) and ALDH activity was measured with flow cytometry. Vitamin D treatment decreased ($p<0.05$) the cisplatin induced increase in OCSCs. Additionally, treatment with vitamin D decreased D ($p<0.05$) spheroid formation compared to cells treated with cisplatin alone or in combination with vitamin D. Collectively, these results demonstrate a role for VDR signaling platinum-induced OCSC conversion. Future work will investigate the impact of cisplatin on VDR signaling, RXR and OCSC plasticity.

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

POSTER #132

HIGH GRADE SEROUS OVARIAN CANCER WITH HSF1 AND MYC COAMPLIFICATION IS SENSITIVE TO ENTINOSTAT TREATMENT

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Ovarian cancer is a disease that affects 1 in 87 women. The most lethal and most common form of this disease is high-grade serous ovarian cancer (HGSOC). This disease is characterized by p53 mutation and copy-number amplifications of oncogenes such as *MYC*, *CCNE1*, and *KRAS*. Despite the high copy-number amplifications of these oncogenes, these amplification events are not used as biomarkers for treatment strategy. The first line treatment for all HGSOC is combination platinum-based chemotherapy and taxol with the only precision medicine option being PARP inhibitors for patients that have BRCA mutations. Not only are there limited treatment options for this disease, but these treatments have a high recurrence frequency. Patients would therefore benefit greatly from using the knowledge of how copy-number amplifications drive HGSOC to find new treatments. One of the most amplified genes in HGSOC is the oncogene *MYC*. This oncogene is amplified along with a neighboring gene *Heat Shock Factor 1* (*HSF1*) in 36% of all HGSOC. Both of these genes are transcription factors that have been shown to have a pro-cancerous transcriptional network. Recent studies have shown that there is cooperation between the two transcription factors in both cancerous and non-cancerous settings. Understanding the interaction between these two transcription factors in the setting of HGSOC and their importance to disease progression for cells with this co-amplification could therefore lead to a treatment strategy of inhibiting HSF1 and MYC. Through a drug screen of inhibitors, we found that cells that carry amplifications of *HSF1* and *MYC* were sensitive to the Histone Deacetylase (HDAC) inhibitor entinostat, which also decreased the levels of HSF1 and MYC protein in these cells. We have shown that knockdown of HSF1 decreased the colony formation ability of these cells, suggesting that HSF1 is not just a passenger gene on the amplicon containing MYC in HGSOC but may be important for carcinogenesis or disease progression. When using the entinostat drug, cell lines with HSF1 and MYC co-amplification lost their HSF1 and MYC protein levels in a dose-dependent manner, suggesting that the mechanisms by which the cells are affected by HDAC inhibition may be through HSF1 and MYC loss. Future studies will investigate whether restoring HSF1 and MYC in these cell lines is enough to prevent entinostat-induced cell death in HGSOC and whether the sensitivity of HGSOC cell lines to entinostat correlates with their HSF1 and MYC expression.

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

POSTER #133

BET INHIBITOR INCREASES DNA DAMAGE, MODULATES WNT SIGNALING, AND SUPPRESSES OSTEOSARCOMA GROWTH IN NAïVE AND METASTATIC DISEASE MODELS

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Osteosarcoma (OS) is an aggressive bone cancer in pediatric adolescent and young adult patients. Survival rate for metastatic and relapsed OS patients remains dismal at <30%. Additionally, no effective standardized salvage therapy currently exists for these patients, in part, due to genomic complexities arising from moderate levels of replication stress (RS). Bromodomain and extra-terminal domain protein inhibitors (BETi) are an underexplored option to target RS for BETi creates an imbalance between transcription-replication kinetics resulting exacerbation of oncogenic RS and cell death. BET proteins are epigenetic readers that play a role in regulating gene expression networks as well as DNA replication and repair. We tested the hypothesis that BET inhibition leads to decreased OS cell growth and potentiates the efficacy of salvage therapy via gene dysregulation and increased DNA damage and RS. Combination index and Bliss independence analyses of bivalent BET inhibitor (BETi) AZD5153 or PROTAC ARV825 in combination with salvage agents demonstrated additive-to-synergistic cell growth inhibition in OS lines. Increased apoptotic-mediated cell death was observed following AZD5153+topotecan. In addition, γ-H2AX levels and comet assays demonstrated that BETi+topotecan induces its effect, in part through increased RS *in vitro*. AZD5153 monotherapy significantly suppressed OS tumor growth in patient-derived xenografts (PDXs) derived from both naïve (PDX96) and metastatic (TT2) OS compared to vehicle ($p<0.05$). Moreover, AZD5153 reduced lung metastatic lesions and increased survival in the 143B OS lung colonization model. *In-vivo* mechanisms of drug-induced tumor response were evaluated. Anti-tumor effect correlated with increased γ-H2AX following AZD5153 exposure in PDX96, indicative of increased RS. RNA-seq analysis and protein validation from vehicle versus BETi-treated PDX96 highlighted dysregulation of several key genes involved in DNA damage response including elevated TXNIP, a tumor suppressor which can induce DNA damage and apoptosis. Downregulation of TCF7, a downstream effector of the Wnt pathway, was also observed following BETi. Global kinome profiling and validation experiments revealed increased levels of EphA2 and EphA4 receptors both implicated in development of drug resistance. Furthermore, in BETi-treated PDX 96 tumors, Wnt/b-catenin pathway activation emerged as tumors rebounded once BETi treatment was stopped. TT2 OS PDX was resistant to commonly used salvage agents ifosfamide and irinotecan; however, combination BETi+topoisomerase inhibitor topotecan increased the probability of survival compared to each agent alone ($p<0.05$) and was well tolerated. These data collectively suggest that BET inhibition alone or in combination with low-dose salvage therapy holds promise as novel treatment strategies in aggressive OS.

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

POSTER #134

TENASCIN C PROMOTES THE OVARIAN CANCER STEM CELL PHENOTYPE WITHIN THE TUMOR NICHE.

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Background:High grade serous ovarian cancer (HGSOC) is the most common, aggressive, and lethal form of ovarian cancer (OC). The greatest challenge affecting treatment outcomes for patients is late, advance-stage diagnosis.In the advanced stages of disease, the critical hallmarks such as metastasis and chemoresistance arepresentand are linked to the ovarian cancer stem cells (OCSCs). OCSCs are known to contribute to the high recurrence of disease and formation of multicellular aggregates (spheroids) during intraperitoneal metastasis. Spheroid formationinvolves coordination of components of the tumor microenvironment (TME), like the extracellular matrix (ECM). Tenascin C (TNC), a secreted ECM glycoprotein is upregulated in HGSOC cells. TNC expression is clinically significant in varying cancer subtypes.However, the functional role of TNC in metastasis and therapy resistancehas not been investigated. We propose to evaluate TNC expression for ovarian cancer progression and therapy resistance both in vitro and in vivo.

Methods: We compared gene expression in OC cells grown as spheroids and monolayers and in chemo-resistant vs chemo-sensitive OC cells.TNC expression was further compared in HGSOC cell lines using real-timequantitative -PCR (RT-qPCR) and Western Blot (WB). TNC knockdown (TNC-KD) using siRNA was evaluated in combination with and withoutcarboplatinto assessspheroid proliferation, colony formation, and expression of stemness related genes, example: Sox-2, Nanog, Oct4 and ALDH1A1. For the in vivo experiments, tumor formation, dimensions, and metastasis were assessed using mice injected with cells transduced for loss of TNC (shTNC) or control (shCTRL) and treated with PBS or carboplatin.

Results:HGSOC cells grown as spheroids have increased TNC expression compared to those grown as monolayers. This pattern is mirrored in chemo resistant cells compared to their related chemo sensitive cells. TNC-KD resulted in decreased spheroid proliferation, colony formation and expression of stemness related genes. Loss of TNC promoted response to carboplatin treatment resulting in further decrease of spheroid proliferation and colony formation. Treatment with recombinant TNC reversed these observations, demonstrating increased TNC expression is important for proliferation and stemness in spheroids. Additionally, OC spheroids treated with the Wnt ligand WNT-3A showed increased TNC expression compared to control untreated cells, as demonstrated by qPCR and WB.Additionally, loss of TNC decreased tumor formation, dimensions, and metastasis in vivo.

Conclusions:TNC increased expression in OC drives stemness associated features such as spheroid formation and chemoresistance. Our data suggests that strategies aimed at interfering with TNC expression and/or secretion along with carboplatin treatment could be a potential combination therapy in OC.

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

POSTER #135

THE HISTONE DEACETYLASE INHIBITOR ROMIDEPSIN IS A POTENTIAL THERAPEUTIC FOR METASTATIC OSTEOSARCOMA

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Osteosarcoma predominately affects adolescents, young adults, and canines. For human patients, the five-year survival rate of those with detectable lung metastases is only 30% while most canine patients with metastatic disease die within 1-2 years. Standard therapy is not effective for this patient group, so it is therefore necessary to identify novel therapies that target the progression of lung 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. The top hits included both histone deacetylase inhibitors (HDIs) that were tested. In follow-up experiments measuring potency on sarcospheres and toxicity on non-transformed cells, romidepsin was the top hit of the 114-drug panel as well of the two additional FDA-approved HDIs and seven HDIs that are in clinical oncology trials. Our goal was therefore to further evaluate romidepsin as a potential therapy for metastatic osteosarcoma. Romidepsin potently decreased viability in sarcospheres from established highly metastatic osteosarcoma cell lines (relative ED50s = 3-36nM). About 50% of the patients (both canine and human) had similar responses to romidepsin (relative ED50s = 1- 18nM) suggesting that those patients might be good candidates for romidepsin therapy. This includes patients who had been heavily pretreated with standard-of-care osteosarcoma chemotherapeutics. Mechanistically, romidepsin blocks proliferation and causes a G2/M cell cycle arrest in fast growing sarcospheres (143B and MG63.3) while in slow growing sarcospheres (LM7 and K7M2) where primarily induces cytotoxicity and there is no cell cycle arrest. Patient-derived sarcospheres are slow growing, so we predict that the primary mechanism will be cytotoxicity. Using a tail vein injection model of osteosarcoma lung metastases, romidepsin dose dependently prolonged survival and reduced the area and number of lung metastases in immunodeficient mice injected with 143B cells and a syngeneic model using K7M2 cells. Romidepsin will be evaluated in mice with metastases from patient-derived cells and if effective, will be repurposed for canine and human clinical trials to improve outcomes in metastatic osteosarcoma without the associated costs and extensive timeframe of traditional drug discovery. Measuring romidepsin responses by patient-derived sarcospheres is a potentially useful way to identify patients likely to respond to romidepsin.

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

POSTER #136

ANCESTRY-STRATIFIED GENOME-WIDE ASSOCIATION META-ANALYSIS OF FLUOROPYRIMIDINE INDUCED TOXICITY

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Background Patients with stage II and III colorectal cancer (CRC) are commonly treated with adjuvant chemotherapy regimens including capecitabine or fluorouracil (5FU). These regimens cause toxicities that vary in severity among patients, and toxicity is more frequent and severe in those with reduced function variants in *DPYD* encoding the metabolizing enzyme, dihydropyrimidine dehydrogenase (DPD). Variation in prevalence of toxicity is thought to be determined by *DPYD* allele frequencies within ancestral populations. In the absence of these known variants, there are severe toxicities that persist. To better under the mechanism of toxicity, we performed an ancestry stratified genome-wide association study meta-analysis.

Methods We performed a meta-analysis across ancestries of a genome-wide association study of 5FU-induced grade 3-4 diarrhea in colorectal patients (n= 4617, dbGaP phs001290). Prior to the meta-analysis, individuals were stratified via ADMIXTURE into European (EUR, n=3841), African (AFR, n =319), East Asian (EAS, n=185), American (AMR, n=149), South Asian (SAS, n=15), and admixed (mixed, n = 108) ancestry groups determined using 1000 Genome Project Phase 3v5 reference panel with duplicate samples removed. Admixed individuals were those who did not have a single superpopulation proportion more than .60 of their global ancestry. Genomes were imputed and phased using TOPMED reference panel via Minimac 4 and Eagle 2.4 (r-squared >0.3). Variants were filtered for genotyping rates >=98%, minor allele frequency >1%, and linkage disequilibrium <80%. Logistic regressions were performed in the stratified groups with the *a priori* covariates sex, age of diagnosis, body surface area, and study site in PLINK 2.0. Inverse variance fixed-effects meta-analysis of stratified results was performed using PLINK 1.9. Significance threshold was Bonferroni corrected for 1,813,574 variants (p-value < 2.8×10^{-08}).

Results. An intronic variant in *UHRF1BP1*, bridge-like lipid transfer protein family member 3A, rs556793499, reached genome-wide significant association with 5FU toxicity (p-value = 1.4×10^{-08}, OR = 7.2). The non-functional variants in *DPYD* were not included due to the low minor allele frequency; however, nine more common variants in *DPYD* had p-values below 0.001. Several other clusters of variants trended towards significance. Prevalence of diarrhea varied significantly by ancestry (SAS = 20.0%, EUR = 11.1%, AMR = 8.1%, EAS = 4.9%, AFR = 4.1 %, mixed = 2.8%, chi-squared p-value = 8.6×10^{-6}).

Conclusion 5FU toxicity varied by genetic ancestry. *UHRF1BP1* appears to be a novel gene associated with 5FU toxicity. Further investigation is needed to determine the mechanism underlying this association with *UHRF1BP1* and genetic ancestry.

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

POSTER #137

ELUCIDATING HOW DNA METHYLTRANSFERASE INHIBITION PREVENTS PLATINUM-INDUCED OVARIAN CANCER STEM CELL ENRICHMENT

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High-grade serous ovarian cancer (HGSOC) is the most common subtype of ovarian cancer (OC). The current standard of care for OC patients is surgery debulking, followed by a combination of platinum- and taxane-based chemotherapies. Despite the high initial response rate to chemotherapies, most patients experience relapse, and the recurrent tumors no longer respond to platinum and other therapies. Relapse and chemoresistance contribute to a low five-year survival rate of less than 30%, making OC the most fatal gynecological cancer. A population of OC cells termed ovarian cancer stem cells (OCSCs) are enriched in recurrent tumors and drive platinum resistance. In addition to OCSCs, recurrent OC tumors also have aberrant promoter DNA hypermethylation, resulting in the silencing of genes involved in tumor suppression and DNA repair, thus allowing OC cells to survive therapeutic assaults. Our lab has shown that pre-treating OC cells with a DNA methyltransferase inhibitor (DNMTi) blocked platinum-induced OCSC enrichment; however, the mechanism behind this observation is still unknown and appears not to be dependent on altering promoter DNA methylation of tumor suppressor genes. RNA-Seq analysis of OC cells treated with platinum with or without DNMTi pre-treatment identifies NF-kB and STAT3 as potential signaling pathways for further examination. NF-kB and STAT3 are transcription factors with known roles in different cellular processes, including stem cell regulation and cancer. My preliminary western blot data demonstrates that NF-kB is activated in response to DNMTi and/or platinum treatment. In contrast, STAT3 is activated by the single treatments but not in the combination. Additionally, NF-kB and STAT3 can work together to regulate gene expression, and activation of STAT3 has been shown to influence NF-kB binding to target genes. My preliminary transcriptional data shows that NF-kB and STAT3 target genes are differentially expressed across the treatments, and among these genes, *GADD45* genes are expressed the highest in the combination treatment group. *GADD45* genes are known for suppressing tumors and promoting differentiation, suggesting their possible roles as potential regulators of OCSC enrichment. Cytokines secreted from cancer cells can also directly regulate cancer stem cells. Media collected from OC cells treated with DNMTi contains increased cytokines regulated via NF-kB compared to media from cells treated with platinum alone. *Together, I hypothesize that DNMTi pre-treatment activates NF-kB but not STAT3, changing NF-kB genomic binding and leading to changes in transcription of target genes and/or cytokine secretion, thus blocking platinum-induced OCSCs.* By elucidating how DNA methyltransferase inhibition blocks platinum-induced OCSC enrichment, this study may provide evidence supporting the use of DNMTi combined with platinum as a new frontline therapeutic strategy for OC.

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

POSTER #138

PIK3C2A AS AN IMMUNOTHERAPEUTIC TARGET FOR TRIPLE-NEGATIVE BREAST CANCER

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Effective tumor antigen presentation is crucial for eliciting strong immune infiltration into solid tumors. However, many types of solid tumors, including triple-negative breast cancer (TNBC), lose their antigen-presenting ability either through mutations of antigen processing and presentation machinery (APM) genes or defective regulation of the endolysosomal network. While APM gene alterations have been extensively studied, little is known about the detrimental consequences of defective regulation of the endolysosomal network on tumor antigen presentation.

The endolysosomal network is tightly regulated by phosphoinositide metabolism, with $PI(3,4)P_2$ being a critical phosphoinositide that regulates receptor endocytosis at the plasma membrane level. In this study, our lab identified a promising target, PIK3C2A, in TNBC tumor cells that, when inhibited or knocked down, significantly enhanced tumor antigen presentation and T cell cytotoxicity. In vitro co-culture experiments demonstrated significant tumor cell killing upon PIK3C2A inhibition. Orthotopic tumor implantation of PIK3C2A-depleted TNBC cells resulted in significant tumor growth suppression in immunocompetent mouse models. By contrast, implantation of these cells in immunocompromised Nu/J mice did not affect tumor growth.

To determine the mechanism by which PIK3C2A modulates tumor antigen presentation, we conducted APM gene screening to assess if tumor antigen presentation was regulated by PIK3C2A at the transcriptional level. Our results indicate that APM genes are not differentially expressed at a significant level, suggesting that the observed enhancement of tumor antigen presentation may not be affected at a transcriptional level. Future directions of this project include comparing the levels of $PI(3,4)P_2$ in control and PIK3C2A-depleted TNBC cells and examining the effects of different $PI(3,4)P_2$ plasma membrane levels on tumor antigen-presenting abilities.

In conclusion, our study has identified a robust immunotherapeutic target that can enhance tumor antigen presentation. Further investigation, particularly in combination with immune checkpoint blockade, is warranted to explore potential synergistic effects.

Translational/Clinical Research ***MD/PhD Student***

POSTER #139

DEVELOPING A LONG-TERM 3D SPHEROID CULTURE PLATFORM FOR TRANSLATIONALLY RELEVANT DRUG REGIMENS

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Long-term culture systems create opportunities to approximate clinical treatment regimens and study development of drug resistance. Compared to monolayers grown on plastic, spheroid cultures have translationally improved cell-cell interactions and morphology, and spheroids intrinsically create diffusion gradients that can model the hypoxic core and drug diffusion of a patient sarcoma tumor. Our lab developed a method of generating spheroids from osteosarcoma cells that is time and cost efficient, highly reproducible, and high throughput, allowing us to investigate novel drugs with increased translatability and rigor over studies using monolayers. Our previous spheroid studies were restricted to 48 hours of culture, making it impossible to model clinical dosing regimens as chemotherapeutics are typically delivered over repeated multi-week cycles. Our objective here is to develop a method for long-term spheroid culture, ultimately to assess the efficacy of novel drugs *in vitro* within translationally relevant treatment regimens. One challenge to sustaining spheroids long-term is in replacing culture media. We generate one spheroid/well in non-adherent 96-well plates to prevent monolayer formation and induce cell-cell aggregation. It is therefore impossible to manually aspirate the media without also aspirating many spheroids. To address this challenge, we took advantage of the adjustable media aspiration/replacement capability provide by the Automated Media Exchange modules of the Agilent Biotek Multiflo FX. Initial experiments identified optimal conditions: an aspiration/replacement flow rate of 10uL/second combined with a 1000g centrifugation prior to each aspiration. These conditions allow us to aspirate/replace 87.5% of the media in each well without removing spheroids. Three cycles of aspiration/replacement therefore exchanges 99.8% of the original media. To determine whether media exchange affects spheroid activity, we measured metabolic activity after media exchange at 6 hours of a 48-hour culture period. This media exchange did not alter metabolic activity assessed by resazurin reduction. To determine the longevity of spheroids, we cultured spheroids for up to 26 days with media exchange every 2 days. The spheroids increased substantially in size during the culture period. To quantify spheroid growth, we measured resazurin reduction at various time points, which increased 5.6-fold during the first 10 days of culture and then remained stable for at least 16 additional days. Osteosarcoma-derived spheroids can therefore be sustained at least 26 days without decline in metabolic activity. These long-term spheroid cultures therefore likely represent growing micrometastases in patients, the leading cause of mortality in many types of cancer. These results also indicate that our spheroid cultures will allow modeling of translationally relevant treatment regimens of novel and standard-of-care drugs, both alone and in combination, and long-term spheroid cultures will allow us to model drug resistance.

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

POSTER #140

CANCER ASSOCIATED FIBROBLASTS SERVE AS A CANCER STEM CELL NICHE AND IMPART CHEMORESISTANCE IN OVARIAN CANCER

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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. $WNT5A^{high}$ patient tumors were shown to have more cancer stem cell markers' expression, which indicated the role of WNT5A in inducing stemness. Correlation analysis in 4 patient datasets showed that $WNT5A^{high}$ patients were associated with a less immune cell population. Deconvolution of ovarian cancer patient data further revealed that the percentage of CAFs in the TME increases with the cancer stage and a significant CAF enrichment in chemo-resistant 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-naïve 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 has shown that WNT5A triggers cancer stem cell enrichment in two ways: 1) stimulating ovarian cancer stem cell self-renewal; and 2) increasing dedifferentiation of bulk ovarian cancer cells. We applied single-cell RNA-seq to identify and characterize the subpopulations of CAFs that induce stemness and chemoresistance, as well as the subpopulations of ovarian cancer cells that are responsive to these signals. 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. By blocking the upstream signaling of WNT5A, we are looking forward to attenuating WNT5A expression in CAFs and preventing ovarian cancer relapse.

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. Targeting this communication to prevent ovarian cancer recurrence can help improve patient outcomes.

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

POSTER #141

TARGETING S-ADENOSYL-METHIONINE (SAM) SYNTHESIS INHIBITS OVARIAN CANCER STEM CELLS

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Ovarian cancer (OC) is the leading cause of gynecologic cancer death. We and others have demonstrated that platinum (Pt)-based chemotherapy can reprogram cancer cells to OC stem cells (OCSCs) and contribute to disease recurrence. Using high-grade serous (HGS) OC cell lines, we demonstrated that cisplatin treatment (IC50, 16hrs) increased ($p<0.01$) the percentage of cells with high aldehyde dehydrogenase (ALDH+) activity, a well-established OCSCs marker, through ALDHFLUOR assay labeling and flow cytometry. As CSCs depend on methionine (Met) metabolism to maintain an undifferentiated state, we investigated the role of Met utilization in OCSCs. HGSOC cells were labeled and sorted into ALDH+/- populations. Expression of Methionine adenosyltransferase 2A (MAT2A), the rate-limiting enzyme in S-adenosyl-methionine (SAM) biosynthesis from Met, was ($p<0.05$) higher in ALDH+ vs. ALDH- cells. Next, we altered Met utilization by reducing extracellular Met level or inhibiting MAT2A by siRNA and inhibitor FIDAS-5. Both approaches inhibited ($p<0.05$) cisplatin-induced increase in %ALDH+ cells and spheroid formation, indicating that Met utilization is essential for platinum-induced enrichment of OCSCs. In addition, MAT2A inhibition downregulated Cyclin E/CDK2, indicating that the G1/S checkpoint regulation in p53-mutant OC had been re-established. To further examine the role of MAT2A in DNA repair, the comet assay was used to assess DNA damage after siRNA inhibition of MAT2A. Based on comet tail length, siMAT2A treatment enhanced ($p<0.01$) cisplatin-induced DNA damage. Moreover, siMAT2A elevated cell death, based on the Annexin-V/propidium iodide apoptosis assay. Given that SAM is the universal methyl-group donor and OCSC enrichment has been associated with aberrant DNA methylation, we investigated the impact of cisplatin and MAT2A inhibition on DNA methylation using methylation specific-PCR (MSP) and a methylation profiling array. MSP analysis demonstrated that inhibition of MAT2A prevented ($p<0.05$) cisplatin-induced CpG island hypermethylation of BRCA1, a tumor suppressor, and ALDH1L1, a folate metabolic enzyme associated with tumor progression. Infinium MethylationEPIC array revealed genome-wide changes in DNA methylation induced by cisplatin and/or siMAT2A. Cisplatin alone increased differentially hypermethylation regions in the siMAT2A compared to the scrambled siRNA. Moreover, the combination of cisplatin and siMAT2A showed a unique pattern of differentially hypomethylated regions compared to cisplatin only, indicating that limiting SAM synthesis altered cisplatin-induced DNA methylation dynamics. Enrichment analysis of the MethylationEPIC results further revealed genes associated with stemness or differentiation. Collectively, these results demonstrated a crucial role of methionine metabolism in Pt-induced OCSC enrichment, providing a better understanding of the mechanism of the interplay between epigenetics and metabolism and novel therapeutic approaches to prevent OCSC enrichment.

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

POSTER #142

CHALLENGING PDAC ADAPTABILITY: UNCOVERING THERAPEUTIC VULNERABILITIES IN APE1 DNA REPAIR MECHANISMS

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Pancreatic ductal adenocarcinoma (PDAC) is a therapeutically challenging malignancy notorious for its resilience to therapeutic strategies and ability to thrive under extreme conditions. The cancer's ability to adaptively resist targeted treatments underscores the need for novel and combinatorial therapeutic approaches. Our research focuses on elucidating the role of APE1/Ref-1, a multifunctional protein involved in PDAC pathobiology. APE1 serves dual key roles: as a redox regulator of transcription factors essential for the proliferation of PDAC cells and as a rate limiting endonuclease in the base excision repair (BER) pathway for DNA repair. While the redox functions of APE1 have been targeted by therapeutic interventions, the development of treatments aimed at its DNA repair function have proven more challenging resulting in comparatively less progress. To advance development in this area and investigate the vulnerability of PDAC cells to impaired APE1-mediated BER, we used CRISPR knock-in editing to engineer PDAC with an E96A change which has been shown to reduce APE1's repair activity. Initial evaluations confirmed the effects of the mutation, revealing a 30-fold reduction in DNA repair endonuclease activity. Surprisingly, phenotypic comparisons between the mutant and wild-type counterparts revealed minimal disparities with respect to growth and proliferation in short term assays. Assessments of potential vulnerabilities to DNA damage, revealed similar findings with the cells exhibiting minimal phenotypic differences in sensitivity when challenged with alkylating agent, MMS, or oxidative DNA damaging agent H2O2. These findings led us to hypothesize that the mutant cells were relying on compensatory DNA repair pathways which were providing a sufficient level of compensation for the decrease in APE1 activity. We further hypothesized that despite these compensatory pathways, the cell lines might still have long-term deficiencies in DNA damage processing which are not initially apparent in cytotoxicity assays. Subsequent colony formation assays revealed that the E96A APE1 mutant cells exhibit poor long-term survival even if only exposed to short term oxidative DNA damage. *In vivo* orthotopic PDAC mouse model experiments further corroborated these findings, with the mutant cells demonstrating a significant reduction in tumor size compared to the WT. While APE1 is the primary endonuclease in the BER pathway, multiple studies have demonstrated APE1-indepent BER in biochemical studies *in vitr*o. We are addressing this hypothesis of APE1-independent repair of oxidative and alkylation damaged sites in DNA through the knockdown of NTHL1, PNKP, and TDP1, proteins implicated in functioning within potential APE1-independent BER pathways. Identifying and characterizing these backup pathways will not only offer a better understanding of APE1's DNA-repair capabilities within PDAC but will also provide a better understanding of how cells can circumvent the reduction of APE1 repair activity. This will allow us to identify potential new therapeutic targets to partner with APE1 inhibition for PDAC treatment.

Translational/Clinical Research ***MD/PhD student***

POSTER #143

GENOMIC ALTERATIONS IN METASTATIC RENAL CELL CARCINOMA (MRCC): IMPACT ON SURVIVAL AND CLINICAL OUTCOMES

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Background

Genomic alterations in RCC can serve as biomarkers for response to therapy. Wild type VHL has shown inferior response to VEGF inhibitors, while patients (pts) with mutated VHL have improved outcomes with immunotherapy (IO)/tyrosine kinase inhibitor (TKI) therapy. We describe the impact of genomic alterations on outcomes and response to front-line therapy.

Methods:

Pts with mRCC who underwent genomic sequencing between 2015-2022 at Indiana University and were treated with front-line therapy were included. Kaplan-Meier method was used to analyze progression free survival (mPFS) and overall survival (OS) using the log rank test to compare groups.

Results:

141 pts were included. Median age was 58yrs (range, 24-81). Tumor histology was 68% clear cell, 10% papillary, 9% unclassified, 1% chromophobe, 1% poorly differentiated, and 9% other. 18% of pts had sarcomatoid features; 13% had rhabdoid. 47% pts had metastasis at diagnosis and 53% at relapse. Metastasis sites were lungs 60%, regional lymph nodes (LNs) 41%, bone 36%, distant LNs 21%, liver 17%, and brain 8%. IMDC risk was 18% good, 48% intermediate, 17% poor, and 17% unknown. Front-line therapy was IO/IO in 21%, IO/TKI in 18%, single-agent TKI in 29%, single-agent IO in 6%, and other in 26%. Genetic alterations included VHL in 50%, PBRM1 in 29%, SETD2 in 23%, BAP1 in 16%, and TP53 in 15%. Median follow-up was 2.74yrs (range 0.1-18.6). Overall mPFS with first line therapy was 1.2yrs (95%CI 0.9-1.5). 2-yr OS was 80% (95%CI: 71-86). For pts with a VHL mutation, mPFS was 1.4yrs (95% CI; 0.9-1.8) compared to 0.9yrs (0.5-1.4) for pts without (p=0.38). 2-yr OS for pts with a VHL mutation was 93% (95% CI: 82-97) vs. 68% (95%CI: 54-78) for pts without (p=0.01). No other mutations impacted overall mPFS or 2-yr OS. mPFS for pts treated with IO/IO was 0.6yrs (95%CI 0.3-0.9) vs. 0.9yrs (95%CI 0.5-1.4) for IO/TKI (p=0.09). 2-yr OS for pts treated with IO/IO was 70% (95% CI 44-85) compared to 94% (95% CI 65-99) for IO/TKI (p=0.29). For pts with SETD2 mutations treated with IO/IO, mPFS was 0.7yrs (95%CI 0.3-1.2) vs. 1.4yrs (95%CI 0.8-1.9) for IO/TKI (p=0.01). For pts with BAP1 mutations treated with IO/IO, mPFS was 0.4yrs (95%CI 0.2-1.2) vs. NE (95%CI 0.2-NE) for IO/TKI (p=0.04). Table 1 outlines outcomes by genomic mutation and therapy.

Conclusions:

VHL mutation was associated with improved 2-yr OS. Pts with SETD2 and BAP1 mutations treated with IO/TKI had improved mPFS, though no differences in OS.

Translational/Clinical Research ***Medical Resident***

POSTER #144

REAL-WORLD DATA WITH ADJUVANT PEMBROLIZUMAB FOR HIGH-RISK RESECTED RENAL CELL CARCINOMA: INSIGHTS INTO CLINICAL OUTCOMES, TOLERABILITY, AND TOXICITY

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Background

In patients with a high risk of recurrence following nephrectomy for renal cell carcinoma (RCC), adjuvant pembrolizumab is associated with longer disease-free survival (DFS) compared to placebo. Uncertainties remain with applying this data to real-world populations, particularly surrounding the overall survival benefit, toxicity rates, and treatment patterns for progressive disease during or after adjuvant treatment. Here, we describe clinical and toxicity outcomes for a real-world population of patients with RCC treated with adjuvant pembrolizumab.

Methods

Patients with RCC who underwent nephrectomy between 2021-2023 at Indiana University and were subsequently initiated on adjuvant pembrolizumab for high-risk disease were included. Kaplan-Meier method was used to analyze recurrence free survival (RFS). Median and 12-mo probabilities were calculated. Recurrence characteristics and subsequent management were also evaluated.

Results

21 patients were included. Median age was 63yrs (range, 39-79). Tumor histology was 76% clear cell, 14% unclassified, and 10% TFE3 translocation associated RCC. 10% of patients had rhabdoid features, and 5% had sarcomatoid features present. Fuhrman grade was grade 2 in 42%, grade 3 in 29%, grade 4 in 29%. 19% of patients received adjuvant pembrolizumab in the M1 NED setting. The median number of adjuvant pembrolizumab doses was 8 (range, 1-17). 86% of patients remained on adjuvant pembrolizumab for ≥3 months, 67% remained on for ≥6 months, 38% remained on for ≥9 months, and 5% completed all 12 months of adjuvant therapy. 33% of patients developed an autoimmune event requiring steroids. 3 patients progressed to metastatic disease- all 3 developed lung metastases, 2 developed liver metastases, 1 developed bone metastases, and 1 also had regional metastatic lymphadenopathy. Of the 3 patients who progressed, 2 were treated with pembrolizumab/lenvatinib and 1 was treated with cabozantinib. At time of last follow-up, all 21 patients were still alive. 12-mo RFS was 95% (95% CI 70-99).

Conclusions

The percentage of patients completing a full 12 months of adjuvant pembrolizumab in this real-world cohort is lower than the 61% completion rate noted in Keynote 564. Adverse event rates appear similar, including rates of autoimmune toxicity requiring steroids.

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

POSTER #145

MIXED ADENONEUROENDOCRINE CARCINOMA OF THE COLON: FROM DIAGNOSIS TO MANAGEMENT

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Introduction: Mixed adenoneuroendocrine carcinoma (MANEC) is a rare malignancy characterized by a combination of adenomatous/carcinomatous cells and poorly differentiated neuroendocrine cells, with each component comprising at least 30% of the tumor. Limited data is available regarding the prevalence and characteristics of colorectal MANEC, primarily relying on case reports and small case series.

Case Description/Methods: A 42-year-old woman presented to the emergency department with abdominal distention, worsening cramping, and severe watery diarrhea. Imaging revealed advanced intra-abdominal malignancy, including extensive hepatic metastasis, peritoneal carcinomatosis with ascites, omental infiltration/nodularity, and suspected peritoneal implants in the pelvis. Malignant wall thickening involving the hepatic flexure of the colon raised concerns of primary colon cancer. A liver biopsy confirmed a diagnosis of MANEC, with 70% small cell neuroendocrine carcinoma and 30% adenocarcinoma. The patient received carboplatin, etoposide, and atezolizumab, yielding a durable response for a few months. A laparoscopic right hemicolectomy with an end ileostomy was performed, but subsequent complications included intraabdominal abscess and colonic perforation. With disease progression, the patient transitioned to folinic acid, fluorouracil, irinotecan hydrochloride, and oxaliplatin (FOLFIRINOX). Despite treatment efforts, the patient's condition deteriorated, and comfort care was chosen.

Discussion: In a retrospective case-matched study by Watanabe et al., the prevalence of MANEC was reported as 3.2% among colorectal disease patients. Due to its dual histological profile, the clinical behavior and management of MANEC significantly differ from adenocarcinoma, primarily influenced by each component's contribution and the neuroendocrine cell type. Complete surgical resection of the primary tumor and metastases remains the mainstay treatment. Adjuvant chemotherapy plays a critical role in improving survival due to the aggressive nature and high recurrence rate of MANEC. The National Comprehensive Cancer Network recommends carboplatin and etoposide or cisplatin and irinotecan as optimal adjuvant chemotherapy for MANEC. Larger studies are needed to evaluate clinical behavior, prognostic factors, survival rates, and effective systemic chemotherapeutic regimens for colorectal MANEC, aiming to establish treatment guidelines for these heterogeneous lesions.

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

POSTER #146

"I COULDN'T TAKE IN THE INFO ALL AT ONCE, THE SHOCK OF DIAGNOSIS WAS SO GREAT": A QUALITATIVE ANALYSIS OF PATIENT EXPERIENCES AND PREFERENCES FOR WHEN TO INTRODUCE OPTIONS FOR OSTEOSARCOMA SURGERY

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Background. In cases where there is no clear best surgical option for osteosarcoma, it is important that families are engaged in shared decision making. However, there is no standard of care for these conversations; options may be discussed at any point from diagnosis to surgery, or not at all. We found no studies specific to the timing of when to have these pivotal discussions. We sought to explore both the patient experience and the ideal patient experience for when to communicate options.

Methods. A two-question survey about when and where to discuss options was disseminated to four Osteosarcoma Facebook Groups. Thematic analysis using open, axial, and selective coding was conducted with two coders; all data was coded twice, independently and for consensus.

Results. There were 52 respondents, primarily parents. Data was thematically organized using a Who, What, When, Where, and Why approach for both questions. These themes were further organized into categories during axial coding.

The overarching theme from the first question exploring the patient experience indicate that most felt unprepared to make a surgical decision. They lacked trust in their surgeon, not all options were mentioned, they lacked information, peer support, and guidance for second opinions. They felt overwhelmed and in shock; it was too much, too fast.

Recommendations.

Who. Improve communication delivery, include other members of the medical team, introduce patient-to-patient peer support: *It would have been nice to discuss surgery options and not just been told what there was.*

What. Include all options, focus on long-term outcomes, provide written resources for options, suggest second opinions: *The conversation should be more in terms of functionality and not framed as "saving the leg."*

When. Stage discussion intentionally, start slowly but early, ongoing, in multiple visits: *Start EARLY - frequent conversations in a variety of settings. There is way too much information to absorb all at once.*

Where. In clinic or in-patient; virtual for follow-up Q&A and second opinions: *We liked discussing this in our surgeon's clinic, because we could calmly look at scans and discuss the surgery and recovery away from oncology.*

Why. Time is limited, diagnosis is traumatic, time to process, tailor to needs: *It is a big, life changing decision.*

Conclusion. This study suggests these conversations are not happening in ways that optimally benefit families. Conversations often occur at diagnosis during an emotional time and families are not feeing supported throughout the decision making process. We aim to develop an evidenced-based timeline for communicating options.

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

POSTER #147

DISPARITIES IN RESPONSE RATES FOR WOMEN AT INCREASED RISK FOR BREAST CANCER AFTER IMPLEMENTATION OF HIGH-RISK SCREENING LAY NAVIGATION PROGRAM

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Introduction: The high-risk screening program (HRSP) at IUSCCC evaluates women at increased risk for breast cancer, providing them with a personalized, guideline-driven plan for prevention including MRI screening, chemoprevention, and genetic testing. High-risk women are identified through risk assessment in mammography, followed by phone contact from a lay navigator. However, retrospective analysis showed low response rates when these high-risk patients were referred to the HRSP. We hypothesize that there will be demographic and socioeconomic differences in those who attend the HRSP versus those who do not.

Methods: This retrospective study analyzed 613 women who were consecutively identified in mammography as being at high risk for breast cancer between 6/2020 and 7/2022. Participants were contacted by the HRSP lay navigator, and response rates were recorded. Demographic information including age, race, zip code, and insurance status were collected through chart review. Those who accepted and attended an appointment were compared to those who did not using Wilcoxon or chi-squared tests. Of the participants who attended their HRSP appointment, we identified the proportion of patients recommended genetic testing, high-risk screening with MRI or chemoprevention who ultimately received those services.

Results: Among the 613 calls made, 22.0% were not reached, 47.1% made an appointment, and 30.0% declined. Out of the 289 participants who made an appointment, 81.7% attended. Those attending the HRSP were significantly younger than those who did not (median age 49 vs. 59, $p<0.001$). Among the 99 contacted participants who identified as Black, 24% accepted and attended an appointment, compared to 39% of White participants (p-value = 0.006).There were no significant differences in uptake of a HRSP appointment based on insurance status or social vulnerability index (by zip code and census data). Of patients ultimately seen in the HRSP, a majority had a BMI of at least 25 (n = 189, 68.3%), were pre/perimenopausal (n=227, 56.4%), and had at least one first-degree relative with breast cancer (n=184, 78.8%). If participants were recommended an MRI, chemoprevention or genetic testing, 76.9% (120/159), 31.1% (38/122) and 71.3% (129/181) of participants, respectively, completed that next step.

Discussion: The HRSP identifies patients at high risk for breast cancer and subsequently aims to provide them with a personalized plan for prevention. However, Black participants were significantly less likely to accept an appointment. In addition, there was a significant portion of participants who declined chemoprevention despite recommendation. These outcomes are important to understand so that we can identify ways to improve participation and further decrease the incidence of breast cancer, particularly in underrepresented minority groups. Achieving this will require further research into the physical, socioeconomic, and psychological barriers to care and how they can be addressed to increase participation in personalized preventative care efforts like the HRSP.

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

POSTER #148

RECURRENCE AND CERVICAL NODAL DISEASE IN PERI-AURICULAR CUTANEOUS SQUAMOUS CELL CARCINOMA UNDERGOING LATERAL TEMPORAL BONE RESECTION

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Objective: Characterize the cervical nodal involvement and recurrence pattern of advanced periauricular cutaneous squamous cell carcinoma (SCC) requiring lateral temporal bone resection (LTBR).

Study Design: Retrospective cohort.

Setting: Single-institution, tertiary referral center

Methods: All patients with SCC of the pinna, external auditory canal, parotid, and peri-auricular scalp undergoing LTBR from 2014-2022 were collected. Patient history, treatments, pathologic reports, adjuvant treatment and follow-up were recorded. Kaplan-Meier and Cox-proportional hazard models were generated to determine predictors of 3-year overall (OS), disease-specific (DSS), and disease-free survival (DFS).

Results: A total of 86 primarily white (98.8%) males (84.9%) with mean age 73.0 years were included. Most tumors were recurrent (64.0%) with a significant portion being radioresistant (20.9%) in immunosuppressed patients (17.4%). Over two-thirds received total auriculectomy, total parotidectomy, and free flap reconstruction, with nearly all patients having levels II (100%) and III (95.3%) neck dissections. Occult cervical nodal disease was identified in 2.7%. Median tumor size was 4.3 cm involving 48.8% and 36.0% having PNI and LVI, respectively. Median follow up was 37.0 months. The 3-year OS, DSS, and DFS were 48.9%, 76.7%, and 68.0%, respectively. The final multivariate Cox-proportional hazard model of 3-year DFS identified immunosuppression (OR=10.96), radioresistant disease (OR=5.44), bone invasion (OR=3.28), tumor size (OR=1.22), and PNI (OR=2.98) as significant predictors.

Conclusions: Occult cervical nodal disease in peri-auricular SCC undergoing LTBR is uncommon. Recurrence is frequent and most at-risk in immunosuppressed patients with advanced, radioresistant tumors with PNI. Trials involving adjuvant or neoadjuvant therapies for these patients are warranted.

Key Words: head and neck cancer; cutaneous squamous cell carcinoma; non-melanomatous skin cancer; lateral temporal bone resection; neck dissection

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

POSTER #149

PERIOPERATIVE RISK FACTORS PREDICTING HARDWARE EXPOSURE AFTER MANDIBULECTOMY AND RECONSTRUCTION

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Objective: Understand the perioperative risk factors that increase the likelihood of hardware exposure following mandibulectomy and mandibular bone reconstruction

Study Design: Retrospective Cohort

Setting: Single-institution, tertiary referral center

Introduction: Mandibulectomy is often necessary for managing tumors, infections, or trauma in the head and neck region. The defect is reconstructed using osteocutaneous free flaps and titanium plates to fixate the bone segments. Unfortunately, postoperative exposure of the mandibular hardware can significantly impair quality of life and usually necessitates extensive revision surgery and reconstruction. We hypothesize that individuals with vasculopathic conditions and other factors that impair wound healing are at a higher risk of experiencing hardware plate exposure following mandibular reconstruction.

Methods: All adults undergoing mandibulectomy and osteocutaneous free flap reconstruction from 2010-2020. Demographics, patient history, surgical parameters, and follow-up data were collected. Comparisons and logistic regression were used to determine factors associated with postoperative hardware exposure.

Results: The cohort consisted of 134 predominantly white (92.5%) male (65.7%) patients with mean age 58.4±13.0 years, 32 (23.9%) of whom had postoperative plate exposure. Between patients with and without exposure, there were no differences observed in demographics, substance use, surgical indications, or reconstructive techniques, neoadjuvant or adjuvant chemoradiation, nor 30-day major surgical site infection. The comorbidities of peripheral vascular disease, hypothyroidism, and immunosuppression were significantly more common in the plate exposure group. In multivariate logistic regression, peripheral vascular disease (OR [95%CI] = 18.8 [1.8-196.3]), hypothyroidism (OR [95%CI] – 3.5 [1.1-10.6]), and immunosuppression (OR [95%CI] = 11.3 [2.4-54.1]) remained significant predictors of post-mandibulectomy hardware exposure.

Conclusion: Patients with pre-existing conditions such as peripheral vascular disease, hypothyroidism, and/or immunosuppression face an increased risk of hardware exposure following mandibulectomy. Recognizing these risk factors is crucial for enhancing the decision-making and counseling process shared between patients and surgeons, and it also directs practitioners to prioritize mitigating these comorbidities before surgery.

Key Words: head and neck cancer; mandibulectomy; osteocutaneous free flap; mandibular reconstruction

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

POSTER #150

REAL WORLD OUTCOMES OF ADJUVANT THERAPY WITH NIVOLUMAB FOR MUSCLE INVASIVE BLADDER CANCER

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Background

In high-risk patients with muscle invasive bladder cancer (MIBC), adjuvant nivolumab after cystectomy was shown to nearly double length of recurrence-free survival (RFS) compared to observation in the Checkmate274 clinical trial. However, there is limited data regarding nivolumab tolerance and clinical outcomes in real world populations. In this study, we describe toxicity rates and disease progression in a real-world population of patients with MIBC treated with adjuvant nivolumab.

Methods

Patients with MIBC who underwent cystectomy at Indiana University Hospital from 2021 to 2023, and were initiated on adjuvant nivolumab for high-risk disease, were included in this study. Baseline demographic and disease characteristics were summarized as median (range) for continuous variables and proportions for categorical variables. The Kaplan-Meier method was used to analyze RFS. The start date used was the surgery date. The progression date was the date of the first line of systemic therapy. For patients who did not go on systemic therapy, they were censored at their death date or last follow-up date. Median, 6 month, and 12 month probabilities were calculated.

Results

In total, 41 patients were included. Median age was 69 years (range 35-84). Tumor histology was 80.5% urothelial, 9.8% mixed (33.2% sarcomatoid and 16.7% micropapillary, plasmacytoid, squamous, and micropapillary urothelial respectively), 4.9% squamous, 2.4% micropapillary, 2.4% unclassified. Variant histology was present in 34.1%. 78% of patients were diagnosed at Stage III, 12.2% at Stage II, and 9.8% at Stage IV. Of these patients, 51.2% did not receive neoadjuvant chemotherapy. The median time on adjuvant nivolumab was 5.3 months (range 0-16.12). Of the total 41 patients included, 78% remained on adjuvant therapy for at least 3 months, 43.9% for at least 6 months, 29.3% at least 9 months, and 7.3% for at least 12 months. Progression and adverse effects were the most common reasons for discontinuation. 7 patients (17.1%) had immune-mediated events 2/2 nivolumab, and 6 patients required steroids. Adverse events that led to discontinuation of nivolumab included dermatitis (in 3 patients), arthritis (3), diarrhea (3), elevated transaminases (2), partial SBO (1), severe fatigue (1), atrial fibrillation (1), lymphedema (1), acute kidney injury (1). 31.7% of patients progressed to metastatic disease. RFS at 6 mo was 92.7% (CI 79-97.6%) and at 12 mo was 82.6% (64.9-91.9%).

Conclusions

Median duration of treatment with adjuvant nivolumab in this study (5.3 months) is lower than duration of treatment in Checkpoint274 (8.8 months). Adverse events leading to discontinuation of adjuvant nivolumab occurred in 17.1% of patients, which aligns with the < 18% noted in Checkpoint274. RFS at 12 months is 82.6%, higher than the 65.1% in Checkpoint274.

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

POSTER #151

TARGETING THE METASTATIC NICHE MEDIATED ACTIVATION OF METASTASIS INITIATING CELLS IN HIGH GRADE SEROUS OVARIAN CANCER

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Extensive metastasis is an important contributor to the high mortality of high grade serous ovarian cancer (HGSOC), the most predominant and lethal subtype of ovarian cancer (OC). However, the mechanisms of regulation of critical steps during HGSOC metastasis is poorly understood and treatment strategies have not been developed to specifically target them. Using a novel organotypic 3D culture model of the human omentum, we have studied the productive crosstalk between metastasizing HGSOC cells and the metastatic microenvironment that is essential for the establishment of metastasis. We have combined this with analysis of matched primary and metastatic tumors from HGSOC patients to determine key regulators of early and advanced metastasis. To identify the clinically relevant microRNAs that can regulate both early and advanced metastasis, we integrated our 3D omentum culture approach with the end point analysis of microRNA expression profiles of matched primary and metastatic tumors from 42 HGSOC patients. Among these, miR-193b emerged as a pivotal regulator, showing consistent downregulation in both early and advanced metastasis stages. The downregulated miR-193b promoted metastatic colonization by enhancing the ability of the HGSOC cells to colonize through the outer layers of the omentum and increased cancer stem cell-like phenotype. Stably overexpressing miR-193b resulted in a significant decrease in metastases in HGSOC xenografts, while stable inhibition increased metastases. Treating a chemo resistant HGSOC patient derived xenograft (PDX) model with miR-193b significantly reduced metastasis. We have identified the microenvironmental signals and the resulting mechanism of miR-193b downregulation through the ERK/EZH2/DNMT1 axis, using heterotypic coculture models, conditioned medium experiments, secretome analysis, inhibition, and rescue experiments. Basic FGF (bFGF) and IGF binding protein 6 (IGFBP6) were the key mesothelial factors that were responsible for the downregulation of miR-193b in cancer cells. They activated ERK within the cancer cells, resulting in the upregulation of EZH2 and DNMT1 expression. Through ChIP and MeDIP, we unveiled that EZH2 instigates H3K27 trimethylation at the miR-193b promoter, recruiting DNMT1 to catalyze DNA hypermethylation. Having elucidated the microenvironment-induced downregulation mechanism of miR-193b, we investigated how its depletion facilitates metastasis. By performing RNA-seq in OC cells overexpressing miR-193b, we identified cyclin D1 (CCND1) as a key target. Knockdown of CCND1 mimicked the decreased expression of stem cells markers (ALDH1A1, OCT4, SOX2 and Nanog), and spheroid formation, caused by miR-193b overexpression. The induction of OC stem cells upon miR-193b inhibition could be rescued by simultaneous overexpression of CCND1. In conclusion, we have uncovered the microenvironment-triggered downregulation mechanism of miR-193b in OC cells, promoting the establishment of metastatic tumors through induction of cancer stem cells via its target CCND1. Treatment of a chemo-resistant HGSOC PDX with miR-193b substantially mitigated metastases, suggesting its potential as a promising therapy for OC patients.

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

POSTER #152

REGULATORY LONG NONCODING RNA OF T CELLS (RELOT) AMELIORATES ALLOGENEIC T CELL-DRIVEN ACUTE GRAFT-VERSUS-HOST DISEASE

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment for high-risk malignant and nonmalignant hematologic disorders. It is often complicated by life-threatening acute graft-versus-host disease (aGVHD). Donor allogeneic (allo) T cells drive aGVHD, yet the underlying mechanisms are incompletely understood. Long noncoding RNAs (lncRNA) are cell-specific regulators of immunity, but their role in allo-T cells is poorly defined. We recently identified a novel T cell-specific lncRNA, *ReLoT*, in peripheral blood T cells as a biomarker for aGVHD and transplant rejection in humans. However, *ReLoT's* biological functions and in vivo role in T-cells remain unknown.

To determine *ReLoT's* in vivo functions, we first established an in vivo overexpression system by adoptively transferring lentivirus-transduced hematopoietic stem cells (HSCs) into B6 mice. Overexpression of *ReLoT* did not impact HSC engraftment, thymic T cell development, nor reconstitution of peripheral (spleen) T cell subsets. Naïve T-cells from *ReLoT* overexpressing mice were then used as donor T cells with WT BM in an MHC-mismatched murine aGVHD model. Overexpression of *ReLoT* in allo-T cells reduced aGVHD severity and mortality ($p=0.027$). We subsequently confirmed these results utilizing a T cell-specific conditional knock-in overexpression system that we developed ($p=0.006$).

Analysis of donor T cell subsets in host spleens post-allogeneic HSCT did not demonstrate differences in naïve, central memory, effector memory, Th1, Th2, nor Th17 subsets between control and *ReLoT* overexpressing donor T cells. Similarly, no differences in proliferation or expression of surface activation markers were observed following in vitro T-cell allo-stimulation. There was also no difference in proliferation, activation of TCR signal transduction molecules, or IL-2 secretion following in vitro anti-CD3/CD28 stimulation. By contrast, the percentage of spleen donor Tregs increased post-allo-HSCT, but this was likely indirect because *ReLoT* overexpression did not alter in vitro Treg differentiation. Together, these data demonstrated that *ReLoT* restrains allo-T cell-driven murine aGVHD and suggested that developing strategies to preserve *ReLoT* expression or enhance its down-stream functions may improve aGVHD-related outcomes.

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

POSTER #153

FUNCTIONAL CHARACTERIZATION OF A RARE CYP3A4 VARIANT IN A PATIENT WITH EXCEPTIONAL CARDIOMYOPATHY DURING SUNITINIB AND AXITINIB THERAPY

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Sunitinib and axitinib can cause cardiomyopathy in up to 30% of patients, which manifests as dyspnea and reduced left ventricular ejection fraction (LVEF). CYP3A4/5 are the primary enzymes responsible for metabolism of sunitinib and axitinib. A 58-year-old female with metastatic renal cell carcinoma experienced severe cardiomyopathy (maximum left ventricular ejection fraction drop of 34%) during sunitinib and later axitinib therapy that was reversed upon drug discontinuation. She provided informed consent and a sample for germline whole genome sequencing (NantOmics GPS Cancer) and targeted pharmacogenomics genotyping at the IU Pharmacogenomics Laboratory. Sequencing data revealed that she was heterozygous for an extremely rare *CYP3A4* variant (rs1483230173; p.P135L) that has not been functionally characterized or curated by PharmVar. The patient's *CYP3A5* genotype was *3/*3. This study's objective was to characterize the function of this rare *CYP3A4* variant.

CYP3A4 was expressed in 293FT cells via transfection of pENTR/D-TOPO vectors containing cDNAs of human *CYP3A4*, *CYB5A*, and *POR*. CYP3A4 activity was quantified using the Promega P450-Glo Luciferin-IPA assay in cells co-transfected with CYB5A and POR along with CYP3A4 plasmids, including the wild-type CYP3A4, *CYP3A4*8* (rs72552799; p.R130Q), *CYP3A4*30* (rs778013004; p.R130X), or the rare *CYP3A4* variant (rs1483230173; p.P135L). This cell-based assay involves a cell-permeable proluciferin substrate that is converted to D-luciferin by CYP3A4 and produces luminescence via reaction with the luciferase detection reagent. Cells expressing the wild-type *CYP3A4* were also treated with 1 μM itraconazole (CYP3A4 inhibitor) as an additional control. All luminescence values, as detected by a microplate reader, were normalized to viable cell count using the Promega CellTiter-Glo 2.0 Cell Viability Assay. All experimental groups were assessed using four biological replicates with 4-5 technical replicates performed for each biological replicate.

The average normalized luminescence, expressed as a percent of wild-type CYP3A4, was 2.3% ± 1.0% (mean ± SD) for *CYP3A4*8*, 0.4% ± 0.3% for *CYP3A4*30*, and 22.0% ± 7.1% for the p.P135L variant. Itraconazole reduced the luminescence in the wild-type CYP3A4 transfected cells by 94% ± 3.5% .

The p.P135L variant appears to be a reduced function CYP3A4 allele. This finding suggests that reduced metabolism of sunitinib and axitinib may have contributed to the patient's cardiomyopathy.

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

POSTER #154

UTILITY OF CIRCULATING TUMOR DNA (CTDNA) AS A PREDICTIVE BIOMARKER FOR DISEASE MONITORING IN PATIENTS WITH NON-SEMINOMATOUS GERM-CELL TUMOR (NSGCT).

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Background: Serum tumor markers (STM) are currently utilized in the management of testicular NSGCT. However, STMs are normal in a substantial proportion of patients with NSGCT (up to 40%) and can be falsely elevated in certain other clinical conditions. Therefore, a highly sensitive and specific biomarker for NSGCT can be helpful for disease monitoring with diagnostic and therapeutic implications. Herein, we test the clinical utility of ctDNA to predict patient outcomes and monitor treatment response in stage II-III NSGCT.

Methods: A total of 106 plasma samples were collected from a cohort of 25 Stage II-III NSGCT patients post-orchiectomy with a median age of 29 years (IQR: 26.8-38.8) and a median follow-up of 10 months (IQR: 7.2-17.5). Longitudinal ctDNA testing was performed using a personalized, tumor-informed ctDNA assay (Signatera^{TM} bespoke mPCR-NGS assay). ctDNA results were analyzed and evaluated for their correlation with clinical outcomes (event-free survival [EFS]) during surveillance after first-line therapy (retroperitoneal lymph node dissection [RPLND] or chemotherapy) or after salvage chemotherapy. EFS is described as the interval from orchiectomy to the date of recurrence or evidence of residual/persistent disease after the completion of RPLND or chemotherapy.

Results: Of 25 patients, 28% (7/25) had stage II and 72% (18/25) had stage III disease. Five patients with stage II disease underwent RPLND, and all tested positive for ctDNA prior to RPLND. Of the 18 patients with stage III disease, 83.3% (15/18) tested ctDNA positive pre-chemotherapy. For patients who completed 1st line treatment with RPLND or chemotherapy and had ctDNA testing post-completion of therapy, none of the patients who tested negative for ctDNA experienced relapse (recurrence rate: 0%, 0/6) compared to a recurrence rate of 30.8% (4/13) in patients who had normal STM levels. The detection of ctDNA was associated with a significantly shorter EFS (HR=11.4, 95%Cl 1.09-1537, log-rank p=0.029) while elevated STM results were not (HR=2.14, 95%Cl 0.51-8.94, p=0.298). ctDNA-positivity remained strongly associated with poor EFS (HR=12.98, 95%CI 1.17-1869, p=0.035) when adjusted for STM status. For patients who progressed after 1st line therapy and were treated with salvage chemotherapy, no events were seen in those who were ctDNA negative after therapy (0/6) compared with an event rate of 25% (2/8) in patients with normal STMs.

Conclusion: Personalized monitoring of ctDNA seems predictive for recurrence in patients with NSGCT. To the best of our knowledge, this is one of the first reports presenting the clinical implications of ctDNA monitoring in this patient population. Our findings suggest that ctDNA monitoring may help optimize clinical decision-making, however, larger prospective studies are needed to validate the findings of this study.

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

POSTER #155

PREDICTORS OF PSA RESPONSE TO LU177-PSMA-617 IN METASTATIC CASTRATION RESISTANT PROSTATE CANCER

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Background: Lu177-PSMA-617 (Lu177) therapy is effective in a subgroup of patients with metastatic castration resistant prostate cancer (mCRPC). We evaluated PSA responses in subgroups of pts with mCRPC.

Methods: Pts with mCRPC who progressed after androgen receptor pathway inhibitor (ARPI) and taxane chemotherapy (or who have refused chemotherapy) were treated with Lu177. Baseline clinical/molecular characteristics and PSMA PET parameters were analyzed along with PSA30 and PSA50 response.

Results: 97 pts were included. Median age 73.4 (55.9, 90.2). At baseline PSMA PET: 78 pts had PSMA + disease in bone, 61 lymph nodes, 9 lungs, 5 liver, and 1 brain. 33 pts had 1 prior ARPI, 64 pts had ≥2 prior ARPI. 13 pts had no prior taxane regimen, 42 had 1 prior taxane, 31 had 2 prior taxanes and 11 had ≥3 taxane regimens. 17 pts had prior PARP inhibitor. With a median follow-up of 6.5 months (1.38-14.54) from starting Lu177 therapy, 56 (57.7%) pts achieved a PSA30 response, and 49 (50.5%) pts achieved a PSA50 response. 59 (60.8%) pts achieved any confirmed PSA response. Grade≥3 toxicity occurred in 9 pts.

Conclusions: There is a trend with higher PSA30 and PSA50 response to Lu177 with higher SUVmean, higher SUVmax, and less prior taxane exposure.

	Number and % of Pts Achieving PSA30 and PSA50 Response			
Characteristic (N)	PSA30 response, N (%)	PSA30 p-value	PSA50 response, N (%)	PSA50 p-value
Location of PSMA + mets				
· Bone (78)	40 (51.3%)	0.009	34 (43.6%)	0.006
· Liver (5)	4 (80%)	0.39	3 (60%)	1.0
· Lymph node (61)	36 (59%)	0.74	32 (52.5%)	0.62
· Lung (9)	8 (88.9%)	0.07	7 (77.8%)	0.16
HRR mutation present (BRCA1, BRCA2, ATM, CHEK2, PALB2, CDK12, FANCA)				
-Yes (29)	16 (55.2%)	0.74	13 (44.8%)	0.46
-No (68)	40 (58.8%)		36 (52.9%)	
Prior ARPI				
-1 (33)	15 (45.5%)	0.03	13 (39.4%)	0.01
-2 (53)	31 (58.5%)		26 (49.1%)	
-≥3 (11)	10 (90.9%)		10 (90.9%)	

Prior taxane regimens				
-0 (13)	10 (76.9%)	0.50	10 (76.9%)	0.14
-1 (42)	24 (57.1%)		22 (52.4%)	
-2 (31)	16 (51.6%)		12 (38.7%)	
-≥3 (11)	6 (54.5%)		5 (45.5%)	
SUVmean quartiles				
-≤7.96 (22)	9 (40.9%)	0.002	7 (31.8%)	0.004
-8.20-11.4 (22)	9 (40.9%)		8 (36.4%)	
-11.6-15.9 (23)	14 (60.9%)		12 (52.2%)	
-≥16.0 (22)	20 (90.9%)		18 (81.8%)	
SUVmax quartiles				
-≤23.0 (23)	8 (34.8%)	0.02	7 (30.4%)	0.04
-24.48-41.0 (23)	11 (47.8%)		9 (39.2%)	
-42.0-63.0 (25)	18 (72%)		15 (60%)	
-≥67.0 (24)	17 (70.8%)		16 (66.7%)	
Number of PSMA + lesions				
-<20 (26)	15 (57.7%)	0.96	13 (50%)	0.88
-20-50 (33)	18(54.5%)		15 (45.5%)	
->50 (33)	19 (57.6%)		17 (51.5%)	
Total lesion uptake quartiles (SUV*mL)				
-≤361 (21)	12 (57.2%)	0.93	10 (47.6%)	0.97
-367-1504.9 (22)	13 (59.1%)		11 (50%)	
-1633-3656.2 (22)	11 (50%)		10 (45.5%)	
-≥3833.37(21)	12 (57.1%)		11 (52.4%)	

POSTER #156

INVESTIGATION OF IMMUNOACTIVE COMPLEXES (IMAX) FOR ACTIVATION OF THE IMMUNE SYSTEM AGAINST MALIGNANT PERIPHERAL NERVE SHEATH TUMORS

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MPNSTs (Malignant Peripheral Nerve Sheath Tumors) are rare spindle-cell soft tissue sarcomas that arise sporadically or from plexiform neurofibromas in patients with Neurofibromatosis Type 1. Surgical resection is the only possibility for curative treatment; however, complete resection is often difficult due to tumor size, metastasis, or location. We intend to employ immunotherapy in treating MPNST. T-cell infiltration in the tumor, PD-L1 expression, and tumor mutational burden are important determinants for immunotherapy success in solid cancers. During disease progression, the T-cell population decreases while $CD4^+$ T regulatory cells (Treg) that mediate immunosuppressive and tolerogenic functions in both homoeostasis and inflammation increases. Also, resident macrophages in the peripheral nerves are activated and play a role in MPNST tumor progression. To reverse the immunosuppressive tumor microenvironment, we use a nanocarrier called IMAX. It consists of a polyethyleneimine-lithocholic acid conjugate (2E'), paclitaxel (PTX), and cyclic dinucleotide (CDN), each playing a distinct role in activating anti-tumor immunity: 2E' carries PTX and CDN, 2E' and PTX induces immunogenic cell death, and 2E' and CDN stimulate antigen presenting cells. IMAX has induced anti-tumor immunity and immune memory in murine colon, breast, and melanoma cancer models. This outcome has led to our hypothesis that IMAX can be an effective therapy against MPNST by activating the immune system against the tumor. Following one dose of IMAX in a murine MPNST cell line model (mNF463a) with Trp53 and Nf1 mutations, tumor regression was observed for 28 days, but tumor regression was not sustained. A second dose of IMAX was administered in this model to determine if long term tumor regression could be achieved. Alongside tumor immune microenvironment phenotyping was performed at day 7 post dosing. We observed a significant increase in infiltration of CD8+ T cells and decrease in Tregs and macrophages in IMAX treated mice. Overall, we conclude that IMAX treatment can transform the MPNST tumor from immune-cold to hot phenotype and the significantly reduced tumor growth may be due to activation of T cells in TME. This work has promised as a groundbreaking approach to maximize the clinical benefits of immunotherapy for MPNST patients.

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

POSTER #157

EXPLORING THE ROLE OF CD24 AND TISSUE TRANSGLUTAMINASES 2 (TG2) IN HIGH GRADE SEROUS OVARIAN CANCER PROGRESSION: IMPLICATIONS FOR TARGETED THERAPY

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Ovarian cancer (OC) poses a significant challenge in oncology due to its high mortality rate and tendency for recurrence. Recent research has spotlighted cancer stem cells (CSCs) as drivers of tumor initiation, progression, and resistance to therapy. Moreover, specific cell surface markers and signaling pathways, including CD24 and tissue transglutaminase 2 (TG2), are increasingly recognized for their pivotal roles in OC biology. CD24, a cell surface protein, serves as a marker for ovarian CSCs and often CD24 overexpression correlates with aggressive tumor poor clinical outcomes and chemoresistance. TG2, an enzyme involved in protein cross-linking and extracellular matrix remodeling, is associated with cancer progression and metastasis. In OC, TG2 expression is linked to aggressiveness and therapeutic resistance, and it plays a role in CSC maintenance and self-renewal.

This study aims to investigate the expression and functional significance of CD24 and TG2 in high-grade serous ovarian cancer (HGSOC) and the key signaling pathways implicated in OC progression. Expression levels of CD24 and TG2 were evaluated in HGSOC cell lines and patient samples in vitro and in vivo. Additionally, the association between CD24 and TG2 was assessed using TG2 blocking peptide (BP) alone and conjugated with CD24 (CD24-BP), comparing their effectiveness against the standard chemotherapy drug, Carboplatin. The effect of BP and CD24-BP on the Wnt signaling pathway, crucial for CSC maintenance, was also explored.

The findings reveals both CD24 and TG2 co-occur in OC. Cells treated with either BP or CD24-BP showed reduced expression of CSCs specific transcription factors such as Nanog, OCT-4 and SOX2 in addition to reduced expression of CSCs specific marker ALDH1A1, LGR5 and CD166. Treatment with BP and CD24-BP significantly reduced the size of the spheroids/tumorispheres as compared to untreated control in OC cell lines and patients derived spheroids. Treated cells showed decreased expression of Wnt-Beta Catenin activated Frizzled class 7 receptor (FZD7) and Wnt-Beta Catenin signaling target gene cMyc. Further, in vivo assay revealed that treatment with BP or CD24-BP reduced the tumor volume and size as compared control.

This investigation uncovers potential crosstalk between CD24 and TG2 signaling pathways, suggesting their cooperative role in driving HGSOC progression. Targeting CSC-specific signaling pathways with TG2 blocking peptide (BP) alone or in combination with CD24 (CD24-BP) could offer promising therapeutic strategies for HGSOC patients. Overall, this study provides novel insights into the involvement of CD24 and TG2 in HGSOC pathogenesis, underscoring their potential as prognostic biomarkers and therapeutic targets. Further research is warranted to elucidate their molecular mechanisms and validate findings in larger patient cohorts, advancing personalized treatment approaches for HGSOC.

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

POSTER #158

DESIGN OF CLINICAL DECISION SUPPORT TO PROMOTE DPYD-GUIDED PRESCRIBING OF INITIAL FLUOROPYRIMIDINE DOSES

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Reduced function *DPYD* variants increase the risk for potentially life-threatening toxicities, even after just one fluoropyrimidine dose. As part of the IU Precision Health Initiative and based on the published guidelines from the Clinical Pharmacogenetics Implementation Consortium and FDA drug labels, IU Health has begun implementing *DPYD* testing and electronic health record (EHR) embedded therapeutic recommendation alerts for patients with clinically actionable *DPYD* variants. The objectives of this study were to (1) evaluate how often prescribers were utilizing *DPYD* testing to guide initial fluoropyrimidine dosing and (2) to develop and implement an EHR-based intervention to facilitate preemptive *DPYD* testing.

Based on EHR data from April 2020 to February 2024, 1134, IU Health patients received *DPYD* testing 276(24%) of them received at least one fluoropyrimidine dose. The most common indications were gastrointestinal (34%), thoracic (32%), and breast (24%) cancers. To determine whether *DPYD* results were available to guide the first fluoropyrimidine dose, we extracted (1) the date/time when *DPYD* results were reported to the EHR and (2) the order date/time of the first fluoropyrimidine dose for each patient. Prescribers **did not** have *DPYD* results at the time of the first fluoropyrimidine order for 197 (71%) of the patients. To determine the genotyping turnaround time, we also extracted from the EHR the order date/time for all *DPYD* tests. The average *DPYD* genotyping turnaround time was 7 ± 3 days [median ± IQR].

Thus, we designed an EHR alert that provides one-click access to the *DPYD* testing order entry; it is triggered upon physician entry of colorectal cancer-related diagnosis codes (i.e., ICD-10 parent codes: C18.X; C19.X; C20.X) for patients who do not have *DPYD* test results in their EHR. Based on a preliminary analysis of data from 18patients, triggering the *DPYD* genetic testing alert upon entry of relevant cancer diagnosis codes would allow 25 ± 28 days [median ± IQR] for *DPYD* results to be returned to the provider through EHR before the first fluoropyrimidine order. The alert is currently undergoing institutional approval. Once approved, it will undergo 6 months of testing, along with refinements, by oncologists treating patients with gastrointestinal cancers. We will also determine if the alerts facilitate the return of *DPYD* results before first fluoropyrimidine prescription.

In conclusion, wedetermined that *DPYD* testing results were frequently not available when the first fluoropyrimidine dose was ordered for cancer patients. Additional health informatic solutions in the EHR are now being developed to better facilitate pharmacogenetic-guided fluoropyrimidine dosing.

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

POSTER #159

SYNERGISTIC EFFECTS OF PARP INHIBITOR RUCAPARIB AND THE NOVEL NQO1 BIOACTIVATABLE DRUG IP-DNQ IN TARGETING NQO1-POSITIVE PANCREATIC CANCER: MECHANISTIC INSIGHTS AND THERAPEUTIC POTENTIAL

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive cancers, for which current treatment strategies are not completely effective, especially in later stages, and most of them lack specificity, leading to harmful effects on normal tissues. NAD(P)H:quinone oxidoreductase 1 (NQO1) is a two-electron oxidoreductase overexpressed in most solid cancers, including pancreatic cancers, and has emerged as a promising target for tumor-selective killing in recent years. Isopentyl-deoxynboquinone (IP-DNQ) is a novel NQO1 bioactivatable drug that has been reported to effectively kill NQO1-positive pancreatic cancer cells. PARP inhibitors are also showing initial success in treating pancreatic tumors, paving the way for further development, and exploring their potential in various clinical scenarios and in combination with other therapies. In this present study, we investigated the combinatorial effect and molecular mechanisms behind IP-DNQ and the PARP inhibitor, Rucaparib, for curing pancreatic cancer. We found that compared to monotherapy, a sublethal concentration of Rucaparib effectively synergizes with IP-DNQ and increases IP-DNQ sensitivity towards $NQO1^+$ pancreatic cancer cell lines. The combination treatment of Rucaparib and IP-DNQ there is an enhanced ROS formation and subsequent DNA damage is observed in MiaPaCa2 cells, even at sublethal doses of IP-DNQ, which is blocked by the NQO1-specific inhibitor dicumarol (DIC). Additionally, PARP inhibition significantly reverses IP-DNQ-induced NAD^+/ATP loss in the combination treatment, which occurs due to IP-DNQ evoked PARP1 hyperactivation. Interestingly, we found that Rucaparib and IP-DNQ combination treatment increases LDH release and IL-1β levels in MiaPaCa2 cells compared to their alone treatments. Further western blot analysis revealed gasdermin E (GSDME) and Caspase 3 cleavage, indicating the induction of pyroptosis by this combination treatment. Furthermore, this combination treatment was also found to be effective for the orthotopic pancreatic xenograft tumor model with enhanced antitumor efficacy and prolonged survival. Pyroptosis is considered an inflammatory programmed cell death, and pancreatic cancers are one of the most immunosuppressive tumor microenvironments. In such a scenario, these novel findings are noteworthy for the development of a treatment strategy for pancreatic cancer cure.

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

POSTER #160

ZNFX1 (NFX1-TYPE ZINC FINGER-CONTAINING 1 PROTEIN) IS A NOVEL TUMOR SUPPRESSOR IN OVARIAN CANCER

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High grade serous cancer is the most common subtype of ovarian cancer. Most patients are diagnosed with aggressive, late-stage disease and have low 5-year survival rate rates. Zinc-finger NFX1-type containing 1 protein (ZNFX1), is a little-studied nucleic-acid sensor belonging to the helicase superfamily. ZNFX1 acts as dsRNA sensor and binds directly to viral RNA to elicit type I IFN response upon viral infection. In ovarian and other cancers, we have shown that ZNFX1 is a key mediator of an epigenetic drug-PARPi combination treatment through mitochondria- and IFN-mediated immune activation. In the current study, it was of interest to further examine ZNFX1 in patient tumors and the functional role in OC cells. By mining the TCGA database, we show that increased ZNFX1 expression tracks with tumor stage and grade in HGSOC. Furthermore, ZNFX1 expression correlated with increased overall survival in a phase 3 trial for therapy-resistant OC patients receiving bevacizumab in combination with chemotherapy. To investigate the functional role of ZNFX1 in OC tumorigenesis in vitro and in vivo, we knocked out ZNFX1 using CRISPR/CAS9 gene editing in BRCA proficient high grade serous OC cells (human, TYK-nu; mouse, KPCA). We observed significant reduction in cell doubling time and increased proliferation, wound healing rate, and migration in the TYK-nu ZNFX1 KO cells compared to the WT. Similarly, in the KPCA cell line, knocking out ZNFX1 resulted in increased proliferation and migration. In both human and mouse cell lines, ZNFX1 KO increased colony and spheroid growth, while having no effect on cell cycle dynamics. Collectively these functional assays provide compelling evidence for a tumor suppressive role of ZNFX1. To further explore the in vivo function of ZNFX1, we injected TYK-nu ZNFX1 WT and KO cells subcutaneously into immunocompromised mice and monitored tumor growth over time. Notably, ZNFX1 knockout resulted in a significant increase in tumor growth compared to the wild type. Immunohistochemical analysis of tumor tissues was carried out. Quantification of the cGAS–STING signaling pathway suggested that ZNFX1 KO tumors had decreased expression of phosphorylated-STING (p-STING) and phosphorylated-TBK1 (p-TBK1), although the differences were not statistically significant due to variation in the individual tumor samples. Increased

expression of the epithelial tumor marker WT1 was observed in ZNFX1 KO samples. Furthermore, ZNFX1 KO tumors, increased expression of the angiogenesis marker CD34 was observed. These findings further support mechanistic involvement of ZNFX1 in STING-dependent inflammasome induction. In summary, in addition to the role of ZNFX1 in initiating pathogen mimicry response in cancer, ZNFX1 acts to suppress cancer cell growth and neoplastic behavior, demonstrating a novel tumor suppressor activity in OC.

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

POSTER #161

DISCOVERY OF SYNTHETIC LETHAL TARGETS TO MYCN IN HINDBRAIN NEUROEPITHELIAL STEM CELLS

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Brain tumors are the leading cause of cancer-related deaths in children, and the most common pediatric malignant brain tumor is medulloblastoma (MB). The Shh-MB subgroup presents the worst prognosis, and additionally is enriched with mutations in the transcription factor MYCN. For decades, MYCN has been challenging to target pharmacologically, due to a lack of enzymatic activity, functional domains, or defined tertiary structure to exploit. One method for indirectly targeting MYCN is to identify synthetic lethal interactions, which has yielded a number of kinase targets in other solid tumor types, suggesting the stability and function of oncogenic MYCN is critically dependent on intracellular kinases. However, these results are not transferable to brain tumors or their progenitor stem cell populations because the biology of MYCN is highly dependent on the cell type. Previous screens used immortalized cell lines from medulloblastoma samples but have lacked a comparable normal control.

Previously, we demonstrated that early-stage neural epithelial stem (NES) cells can be derived from embryonic and/or induced pluripotent stem cells. Previous studies have demonstrated that these cells represent progenitors in the hindbrain neuroepithelium at 5-7 weeks gestation. NES cells can be reliably and efficiently derived directly from the hindbrain at this developmental stage, and these cells retain the neuroepithelial stage characteristics (including neural rosette markers SOX1, PLZF, and DACH1), and hindbrain identity. Importantly, they retain the developmental potency for cerebellar cells, including granule cells, the putative cells of origin for Shh-MB. Hindbrain NES cells therefore provide an ideal human system to model cerebellar development, and diseases that arise from these progenitors such as medulloblastoma.

This study targets overexpression of MYCN in NES cells to uncover unique synthetic lethal vulnerabilities and to set the stage for the development of novel drugs targeting medulloblastoma-initiating stem cells. It demonstrates the feasibility of a large-scale CRISPR-Cas9 synthetic lethal screen in NES cells. By comparing lethal targets in cells artificially overexpressing MYCN to those in their wild-type counterparts, this study presents potential pharmacological targets within the human kinome with which Shh-MB cells can be eliminated while preserving non-cancerous cells.

Results of the synthetic lethal screen have presented us with 26 potential kinase targets. Based on the existing availability of approved kinase inhibitors, we have initially selected Bruton's tyrosine kinase (BTK) inhibitors such as Zanubrutinib for *in vitro* studies. Future studies include kinase activity assays in both MYCN-overexpressing and wild-type cell lines, as well as establishing dose-response curves for various kinase inhibitors in both, to determine the practical viability of potential treatments.

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

POSTER #162

REVEALING THERAPEUTIC VULNERABILITIES TARGETING TFCP2-REARRANGED RHABDOMYOSARCOMA

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Rhabdomyosarcomas (RMS) are the most common soft tissue sarcomas in children and adolescents, and young adults. These mesenchymal malignancies arise from connective tissues and exhibit extensive heterogeneity with new subsets being continually characterized. Front-line multimodal therapy, which has not improved in the last 30 years, often fails in patients with aggressive RMS disease. Primary intraosseous RMS (IORMS) is a recently classified, rare, aggressive, spindle cell-like RMS subset, which has been distinguished by specific gene fusions, including FUS-TFCP2. This fusion protein blocks myogenic differentiation, induces transcription of Anaplastic Lymphoma Kinase (ALK) and Telomerase Reverse Transcriptase (TERT), and inhibits DNA repair. It is also associated with poor prognosis, frequent metastasis, and has no effective systemic therapy. Although case reports of treatment with ALK inhibitors (ALKi) have shown modest results, resistance always emerges. The ability to understand disease pathogenesis and identify therapeutic targets for rare RMS subsets is hindered by the lack of appropriate pre-clinical in-vivo models. Thus, there is a critical need for developing such models to identify therapeutic options that will block disease progression, enhance quality of life, and ultimately achieve a cure for these patients.

In collaboration with the Pediatric Cancer Precision Genomics Program at the Riley Hospital for Children, we recently developed a set of FUS-TFCP2 fusion-containing IORMS patient-derived xenografts (PDX) from the same patient at different therapeutic timepoints. PDX174 was derived prior to ALKi treatment and PDX199 from the patient following disease progression after 16 months of therapy with ALKi, lorlatinib. Consistent with IORMS characterization, the PDX174 transcriptome shows significantly increased expression of ALK and TERT, 10.6- and 14.8-fold respectively, compared to normal human skeletal muscle cells. ALK protein overexpression was confirmed by western blot but was notably absent in the subsequent PDX199 model. To further investigate the PDX174 model, we conducted an in-vivo screen of six different small molecule inhibitors (SMIs) based on previously prioritized biomarkers of therapeutic response. BET inhibitor (BETi), AZD5153, resulted in a complete block in tumor growth ($p<0.05$) compared to the vehicle and other SMIs. However, resistance did emerge after four weeks of AZD5153 treatment. In contrast, while CDK4/6 inhibitor, palbociclib, and CHK1 inhibitor, SRA737, showed moderate inhibition of tumor growth, drug resistance quickly followed within 2 weeks. Global kinome analysis, using multiplexed inhibitory bead binding and mass spectrometry, indicated a significant increase in the mitogen- and stress-activated kinase 1 in AZD5153-treated tumors compared to the vehicle group, revealing a potential therapeutic vulnerability in the BETi-resistant tumors. Detailed interrogation of drug response in models such as PDX174/PDX199, which reveal actionable molecular signatures before and after targeted therapy, will increase our mechanistic understanding of tumor adaptive responses. This will aid in designing therapies that mitigate the emergence of therapeutic resistance that occurs in IORMS.

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

POSTER #163

TUMORIGENICITY FROM MUTANT STEM CELL PARTICIPATION IN WOUND HEALING

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While surgical intervention remainsa viable approach for many solid tumors, accumulating evidence suggests that the surgical procedure itself may paradoxically contribute to tumor progression and metastasis in certain cases. The tissue injury associated with tumor resection elicits a wound healing response, which, when dysregulated, may create a permissive microenvironment for residual tumor cells to thrive. However, the precise mechanisms by which the wound healing process promotes tumor recurrence and metastasis remain poorly understood, representing a critical knowledge gap. Therefore, this study aims to elucidate the mechanisms that trigger tumor formation from transformed epithelial stem cells when they participate in the wound healing process.

The 4-nitroquinoline 1-oxide (4NQO) carcinogen model is widely used to induce head and neck squamous cell carcinoma (HNSCC) cancer in rodents. Both published data and our prior research indicate that 4NQO predominantly triggers tumors in the tongue, with the hard palate showing natural resistance to 4NQO-induced carcinogenesis. This aversion makes it an ideal model for investigating the role of wounding in promoting tumor formation. Our hypothesis is that mutant epithelial stem cells, when engaged in the wound healing process, can initiate tumor formation. To test this hypothesis, we induced mice with 4NQO and subsequently challenged them with wounds in the hard palates. Our findings revealed a significant increase in tumor formation in 4NQO-treated mice with wounded hard palates compared to their unwounded counterparts. Although mutant epithelial stem cells demonstrated higher rates of proliferation during wound healing, the overall wound healing rate was reduced compared to intact tissue, likely due to abnormal epithelial tissue formation. Notably, we observed infiltration of T cells and macrophages in the epithelial layer of the wounded normal epithelium. Yet, with mutant skin stem cells present, we found the unusual entry of additional B cells and neutrophils. The disruption of the basement membrane and the loss of epithelial-connective tissue borders during wound healing facilitate this aberrant immune cell infiltration.

In summary, our findings suggest that the wound healing microenvironment significantly influences the fate of mutant epithelial stem cells and potentially provides a favorable environment for tumor initiation. Wounds significantly promote tumor formation when epithelial cells are predisposed to malignant transformation, due to tissue architectural disruption, aberrant immune cell infiltration, and proliferative cues from the wound healing microenvironment. This study sheds light on the interplay between wound healing, mutant epithelial stem cells, and tumor initiation, highlighting the potential risks associated with surgical interventions in individuals with preexisting epithelial mutations. These findings may inform strategies to modulate the wound healing microenvironment and mitigate the risk of tumor formation following surgical procedures or injuries.

Basic Science ***Research Technician***

POSTER #164

STAGING ACCURACY AND TREATMENT SEQUENCING FOR CLINICAL T2N0 GASTRIC CANCER

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Background: Guidelines recommend that patients with clinically staged T2N0 gastric adenocarcinoma undergo either neoadjuvant chemotherapy or upfront resection. Patients with pathologically confirmed T2N0 disease after upfront resection can be observed without chemotherapy. These guidelines create the possibility of both systemic therapy overuse (i.e., patients with true T2N0 disease receiving preoperative chemotherapy) and underuse (i.e., failure to receive postoperative chemotherapy after upstaging) depending on clinical staging accuracy. Our objectives were to 1) define the rate of upstaging after upfront resection, 2) identify factors associated with upstaging, and 3) describe association between postoperative treatments and survival with and without upstaging.

Methods: The National Cancer Database was used to identify patients with clinical T2N0 gastric adenocarcinoma. Factors associated with upstaging after upfront surgery were assessed using multivariable logistic regression. Postoperative survival was assessed using multivariable Cox proportional hazard models.

Results: Of 4093 patients undergoing upfront resection for clinical T2N0 gastric cancer, 1952 (47.7%) were pathologically upstaged. Patients were more likely to be upstaged if they were male (aOR 1.21, 95%CI 1.05-1.41; P=0.009), had >3.0cm tumors (aOR 2.31, 95%CI 1.98-2.70; P<0.001), or had poorly differentiated tumors (aOR 2.22, 95%CI 1.90-2.60; P<0.001). Patients were less likely to be upstaged if they were ≥80 years old (aOR 0.65, 95%CI 0.49-0.87; P=0.004) or had distal tumors (aOR 0.77, 95%CI 0.66-0.94; P=0.008). Of 1952 patients who were pathologically upstaged, 1124 (57.0%) received postoperative chemotherapy which was associated with improved survival in adjusted models (HR 0.65, 95%CI 0.50-0.84; P=0.001). Of 2141 patients with pathologically confirmed T2N0 or less disease, 250 (11.7%) received postoperative chemotherapy which was not associated with improved survival (HR 0.96, 95%CI 0.68-1.38; P=0.860).

Conclusions: Pathologic upstaging after upfront resection in patients with clinical T2N0 gastric cancer is associated with certain patient and tumor characteristics. Postoperative chemotherapy is associated with improved survival in patients who are pathologically upstaged at resection, but not in those with pathologically confirmed T2N0 or less disease. An upfront surgical approach may be preferred in select patients, especially if avoiding chemotherapy is desired. The possibility of overtreatment with a neoadjuvant approach and suboptimal treatment after upfront resection should be discussed during treatment planning.

Translational/Clinical Research ***Resident***

POSTER #166

SAFETY OF PEGFILGRASTIM IN PATIENTS WITH METASTATIC GERM-CELL TUMOR RECEIVING COMBINATION CHEMOTHERAPY CONTAINING BLEOMYCIN

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Introduction

There are conflicting reports on the impact of growth factor support in combination with bleomycin on the development of bleomycin associated lung toxicity in various malignancies. In addition, theories remain surrounding the possibility of worsened hematologic toxicity when receiving pegfilgrastim too close to the time of subsequent chemotherapy that is given in consecutive weeks. Here, we aim to evaluate the safety of the use of pegfilgrastim in patients with germ-cell tumor (GCT) undergoing combination chemotherapy containing bleomycin.

Methods

The prospectively maintained Indiana University testicular cancer database was queried for patients with metastatic GCT treated between 2010-2023 at Indiana University with chemotherapy containing bleomycin. Safety in patients who received at least one dose of pegfilgrastim were compared to patients who did not receive any doses. Bleomycin lung toxicity was per the discretion of treating physician. Comparison between groups was done using Chi-square tests for categorical variables or Wilcoxon test for continuous variables.

Results

196 patients met eligibility. Median age at diagnosis was 26.7 (range, 20.2-50). Primary site was testis in 95.4%, retroperitoneum in 2.6%, and mediastinum in 2%. 79% of patients included had nonseminomatous germ-cell tumor (NSGCT). IGCCCG risk was good in 146, intermediate in 19, and poor in 31 patients. First-line chemo was BEPX3 in 71% of patients, BEPX3 with EPX1 in 9% of pts, BEPX4 in 10% of patients, and other in 10% of patients. There were 49 patients (25%) who received at least one dose of pegfilgrastim with chemotherapy. There was no statistically significant difference in the type of chemotherapy received, metastatic sites of disease at time of diagnosis, or IGCCCG risk in patients who received pegfilgrastim vs. those who did not. The median number of pegfilgrastim doses received was 2. The median number of doses of bleomycin received was 9 for both patients who received pegfilgrastim and those who did not. In those who received pegfilgrastim, 4 patients developed bleomycin lung toxicity vs. 13 patients who did not receive pegfilgrastim ($p=1.000$). Of these 17 pts, 9 were diagnosed on symptoms and exam alone, 2 by imaging with symptoms, 2 by imaging without symptoms, 3 on PFTs ordered because of symptoms, and 1 on PFTs alone without symptoms. 3 patients required steroids. There were no fatalities related to pulmonary toxicity. Median nadir ANC in those who received pegfilgrastim was 3350 vs. 800 in those who did not ($p<0.0001$).

Discussion

The administration of pegfilgrastim with combination chemotherapy containing bleomycin for metastatic GCT did not increase the risk for bleomycin associated lung toxicity. Hematologic toxicity, demonstrated by nadir ANC, was worse in pts who did not receive pegfilgrastim.

Translational/Clinical Research ***Resident***

POSTER #167

SELF-SUPERVISED DETERMINATION OF GLIOMA IDH MUTATION STATUS FROM H&E-STAINED WHOLE SLIDE IMAGES

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Introduction: Infiltrating gliomas are the predominant primary adult brain tumors. Isocitrate-dehydrogenase (*IDH*) mutational status is diagnostically critical, with prognostic and therapeutic implications, but only determined after molecular analysis. Here we seek an interpretable computational predictor of *IDH* status from H&E-stained whole slide images (WSI).

Material and methods: We identified 1,534 WSI (756 *IDH*-wildtype, 778 *IDH*-mutant) from TCGA-LGG and TCGA-GBM, and 114 WSI (82 *IDH*-wildtype, 32 *IDH*-mutant) from the University of Pennsylvania (UPenn). 20X magnification WSI underwent comprehensive curation and tiling into 256x256 patches. Features were extracted using pre-trained i) ImageNet weights as baseline, and ii) self-supervised vision transformer(SSL-ViT). A weakly-supervised attention-based multiple-instance-learning framework distinguished WSI between *IDH*-wildtype or *IDH*-mutant, while generating attention heatmaps for visual interpretation. Performance was initially evaluated as 10-fold cross-validation (CV) across TCGA data partitioned in training (80%), validation (10%), and test (10%) sets. Independent evaluation conducted on the unseen hold-out UPenn data.

Results: Evaluation on TCGA and UPenn data yielded accuracy of 88.8% (10-fold-CV AUC = 0.955) and 92.6% (Hold-out data AUC=0.976), respectively, with high sensitivity, i.e., confidence in predicting IDH-mutant. The SSL-ViT model on hold-out data demonstrated superior accuracy and AUC to the baseline, by 6.7% and 10.8% improvements, respectively. Heatmap assessment indicated IDH-wildtype tumors exhibit distinct regions of significant pleomorphism and microvascular proliferation, while IDH-mutant tumors exhibit dense nodular cell concentrations, microcystic architecture, uniform gemistocytic cells, and fibrillary background areas.

Conclusion: Our accurate H&E-based computational determination of glioma IDH status, with interpretations aligned with human-identifiable features, can obviate the need for molecular analysis and enable expedited diagnosis even in community settings.

Translational/Clinical Research ***Staff***

POSTER #169

DEVELOPING METHODS TO QUANTIFY HUMAN TUMOR BURDEN IN MOUSE MODELS

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Many cancer researchers perform in vivo experiments using a murine model. This includes using both xenograft and syngeneic models. To interpret data from these models, it is necessary to quantify the amount of tumor burden in these animals, which can be challenging to do in a consistent and non-subjective way. One of the methods to analyze the amount of tumor burden is by using conventional histological analysis, which can be subjective. Therefore, our lab's goal was to develop standardized methods for quantifying tumor burden in our in vivo models. Our lab uses a lung metastatic model of osteosarcoma. We have developed a method to standardize histological analysis of mouse lungs with traditional hematoxylin and eosin staining. The method contains a specific rule set for quantifying number of metastases and metastatic area within mouse lungs. This method has been validated by comparing results between multiple raters including students at different levels (graduate, undergraduate, high school). For both number of metastases and metastatic area analyses, we plotted the results from two different students on the same graph and did a linear regression analysis. For both students, the lines had R^2 values of 0.8-0.9, indicating that the relationship between the two student's analyses were close to linear and therefore very similar. Our lab also developed a species-specific qPCR method that can be used to detect the amount of human DNA within a tumor sample. This method uses primers for a human-specific gene (beta-actin) and a mouse-specific gene (myoglobulin) to detect the amount of human DNA and mouse DNA within a sample based on standard curves with known amounts of human and mouse DNA. Specifically, our lab used a metastatic model of osteosarcoma and detected the amount of human DNA in mouse lungs. Primer efficiencies for this method are within 80-100%. Results from this qPCR method parallel the results from our histological analysis, validating our qPCR method. We were able to determine a dose dependent effect of our novel therapeutic in mouse lungs using these two methods. In addition, we can use this method to identify the percentage of human and mouse DNA within a patient-derived xenograft (PDX) sample. This allowed us to identify a PDX sample that was primarily mouse cells rather than a mixture of mouse and human. Having reliable methods to quantify tumor burden in mouse lungs will help researchers who are studying osteosarcoma, but these methods can also be applied to other cancers and a variety of tumor models including those in organs other than lung and PDX models. Future directions for our work will investigate a method to use for a syngeneic mouse model to quantify the amount of mouse cancer cells within mouse lungs.

Translational/Clinical Research ***Undergraduate Student***

POSTER # 170

THE NAE1 INHIBITOR MLN4924 SENSITIZES BOTH BRCA1 WILD TYPE AND MUTANT TRIPLE-NEGATIVE BREAST CANCER TO CISPLATIN BY REDUCING THE NUCLEOTIDE EXCISION REPAIR PROTEIN XPC LEVEL

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Background: Triple-negative breast cancer (TNBC) shows poor survival and prognosis, high recurrence, and poor response to conventional therapeutics. To develop novel therapeutics for TNBC, we explored the efficacy of the neddylation inhibitor, MLN4924. We showed that MLN4924 displayed increased sensitivity against TNBC compared to non-TNBC subtypes. Mechanistically, MLN4924 induced extensive re-replication and DNA damage leading to cell death. Furthermore, MLN4924 improved cisplatin efficacy by elevating the DNA damage levels. To demonstrate the mechanisms of MLN4924+cisplatin-mediated enhanced cell death, we examined the XPC level, a key factor in the nucleotide excision repair (NER) pathway, playing a central role in cisplatin damage repair. Neddylation of CUL4A promotes XPC ubiquitination which influences XPC binding to the DNA damage sites for NER.

Methods: We examined the XPC protein level by Western blot assay and RNA level by Real Time PCR using both BRCA1-wild type (MDA-MB-231) and BRCA1-mutated (MDA-MB-436) cell lines and mouse xenograft tumor samples. We evaluated if the proteosome inhibitor MG132 stabilizes XPC ubiquitination and protein levels by treating these cells with MG132 for various times. We also overexpressed XPC in these cell lines and examined the effect on survival upon MLN4924 and MLN4924+cisplatin treatments.

Results: Our results demonstrate that MLN4924 reduces XPC ubiquitination upon shorter drug treatment but reduces the protein level upon longer drug treatment in both BRCA1-wild type and BRCA1-mutated cell lines. MG132 treatment did not stabilize the XPC ubiquitination level upon shorter drug treatment but stabilized the protein level upon longer drug treatment. We also observed a reduction in the XPC protein level in tumor samples which correlated with the increase in the DNA damage level. Surprisingly, XPC overexpression improved the cell survival in both MLN4924- and MLN4924+cisplatin-treated BRCA1-wild type and mutated TNBC cells.

Conclusion: We hypothesize that a reduction in the XPC ubiquitination and protein levels plays a role in cisplatin-induced DNA damage repair reducing NER efficiency and consequently increasing the DNA damage upon MLN4924+cisplatin treatment. Since overexpression of XPC results in improved survival of both MLN4924-and MLN4924+cisplatin-treated cells, we anticipate that XPC not only regulates cisplatin damage repair through NER but may have a broader role in MLN4924 sensitization beyond the NER pathway. Importantly, MLN4924 treatment does not affect the XPC mRNA level emphasizing that neddylation influences the XPC protein level. In summary, our research uncovers novel insights into the role of XPC in both MLN4924 and MLN4924+cisplatin sensitization, shedding light on potential avenues for innovative therapeutic strategies for TNBC patients.

Translational/Clinical Research ***Assistant Research Professor***

POSTER # 171

EVALUATING THE ACCURACY OF PHARMACOGENOTYPE EXTRACTION FROM SOMATIC WHOLE EXOME SEQUENCING

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Background

Pharmacogenomics (PGx) testing can help reduce toxicities and improve efficacy of several drugs used to treat cancer and associated symptoms. PGx results can be determined from germline whole-exome sequencing (WES), but somatic mutations may cause discordance between tumor and germline DNA. Since clinical diagnostic sequencing in oncology frequently only includes tumor DNA, there would be clinical value in calling germline PGx genotypes from tumor DNA. Thus, the goal of this study was to assess the feasibility of using somatic WES data to call germline PGx genotypes.

Methods

Germline and somatic WES data were obtained as part of the clinical workflow for 719 patients treated at the Indiana University Precision Genomics Clinic. Aldy v3.3 was implemented using the default settings in LifeOmic's Precision Health Cloud™ to call PGx genotypes from somatic WES. Somatic Aldy calls were compared with previously validated Aldy germline calls for 13 genes: CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, CYP4F2, DPYD, G6PD, NUDT15, SLCO1B1 and TPMT. Somatic read depth was >100x, except for the intronic CYP3A4*22 variant, which was >30x.

Results

Somatic and germline Aldy calls were compared. Based on 9347 genotypes, 781 (8%) calls were discordant. Discordant calls were most common in CYP2B6 (26%) and CYP2D6 (24%), followed by SLCO1B1 (23%), DPYD (10%), and CYP2C19 (9%). In contrast, all Aldy calls for G6PD and NUDT15 were concordant. Discordant calls for at least one variant were observed in 449 (62%) of the 719 individuals. Colorectal (12%), breast (12%), prostate (6%), and non-small cell lung (6%), were the most common first cancer diagnoses in our group, and the frequencies of discordant Aldy calls did not appear to differ by cancer type ($p>0.05$).

Conclusion

Pharmacogenetic genotyping from somatic tumor DNA may be useful as a screening tool; however, additional work needs to be done to determine if the remaining discordant calls can be corrected by modifying the informatics tools or if they are due to somatic mutations.

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