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POSTER #1

KRASG12D INHIBITION WITH MRTX1133 EXHIBITS POTENT ANTITUMOR ACTIVITY AND ENHANCES NAB-PACLITAXEL-BASED CHEMOTHERAPY RESPONSE IN PANCREATIC CANCER

Mitchel Ramos[1], Nicola Grimaldi[2], Niranjan Awasthi[1]

[1] *Indiana University School of Medicine-South Bend*
[2] *Department of Neuroscience and Behavior, University of Notre Dame, South Bend, IN*

Email: *mitramos@iu.edu*

Background: Pancreatic ductal adenocarcinoma (PDAC) remains one of the most challenging malignancies to treat, with a poor prognosis. Nab-paclitaxel plus gemcitabine (NPT-GEM) represents the standard treatment, providing a median survival of approximately 8.5 months. Genetic studies have identified *KRAS, TP53, p16/CDKN2A*, and *SMAD4*as the most common mutated genes in PDAC, with *KRAS* mutations present in over 90% of cases, driving tumorigenesis. The predominant *KRAS*-mutation subtypes in PDAC include G12D (~49%), G12V (~30%), and G12R (~12%). Recent therapeutic strategies targeting KRAS-driven tumors focus on mutation-specific inhibitors, such as selective $KRAS^{G12C}$ inhibitors and pan-KRAS inhibitors. While $KRAS^{G12C}$ inhibitors have shown promise in non-small cell lung cancer harboring $KRAS^{G12C}$ mutations, they have limited applicability in PDAC, where $KRAS^{G12D}$, $KRAS^{G12V}$, and $KRAS^{G12R}$ mutations are more prevalent. MRTX1133 is a $KRAS^{G12D}$ inhibitor that targets the $KRAS^{G12D}$ mutation in solid tumors by reversibly binding to the $KRAS^{G12D}$ protein, preventing its activation and downstream signaling. We hypothesize that MRTX1133 will exhibit significant antitumor activity alone and in combination with standard chemotherapy in PDAC.

Methods: The *in vitro* proliferation of $KRAS^{G12D}$-mutant (AsPC-1, HPAF-II, SW-1990), $KRAS^{G12C}$-mutant (MiaPaCa-2) and $KRAS^{wt}$ (BXPC-3) cells was assessed using the colorimetric WST-1 assay. Protein expression was analyzed by Immunoblotting. Tumor growth studies were conducted using NOD/SCID mice bearing AsPC-1 subcutaneous xenografts.

Results: *In vitro* assays demonstrated dose-dependent inhibition of proliferation in $KRAS^{G12D}$ -mutant cells with both NPT-GEM and MRTX1133, with an enhanced inhibitory effect observed when used in combination. MRTX1133 alone had minimal effect on $KRAS^{G12C}$-mutant (MiaPaCa-2) and $KRAS^{wt}$ (BxPC-3) cells. Immunoblot analysis of $KRAS^{G12D}$-mutant AsPC-1 and HPAF-II cells revealed decreased expression of phosphor-RAF, phospho-ERK, phospho-AKT, phospho-S6 and phospho-MEK, alongside increased expression of apoptosis markers cleaved-PARP-1 and cleaved-caspase-3. *In vivo* tumor growth inhibition studies demonstrated significant tumor growth delay with NPT-GEM and MRTX1133, with the combination therapy exhibiting the greatest tumor regression. Compared to the control group (327 mm^3), average tumor volume was reduced to 129 mm^3 with NPT-GEM, 56 mm^3 with MRTX1133, and -4 mm^3 (tumor regression) with the combination therapy. At the end of the treatment period, average tumor weight was 0.36 g in controls, 0.17 g with NPT-GEM, 0.1 g with MRTX1133, and 0.06 g with combination therapy. There was no significant change in mouse body weight during the treatment period, indicating no discernible treatment-related toxicity in any group.

Conclusion: MRTX1133 exhibited significant antitumor activity in $KRAS^{G12D}$-mutant PDAC preclinical models and enhanced the response to standard chemotherapy. These findings highlight the therapeutic potential of MRTX1133 for the clinical management of $KRAS^{G12D}$-mutant PDAC patients.

Basic Science ***Faculty***

POSTER #2

INHIBITORY EFFECTS OF ORAL BACTERIAL SPHINGOLIPIDS ON ORAL SQUAMOUS CELL CARCINOMA

Chiaki Yamada[2], Alexandru Movila[1]

[1] *Indiana University School of Dentistry, Indianapolis, IN*
[2] *Indiana University School of Dentistry*

Email: *chiyama@iu.edu*

Objectives: Oral squamous cell carcinoma (OSCC) is one of the most common oral cancers in the oral cavity and lip. It is still elusive whether the key periodontal pathogen, *Porphyromonas gingivalis* (*P. gingivalis*), possesses anti-cancer effects. Acid ceramidase (aCDase/ASAH1) regulates the balance between tumor-suppressing ceramides and tumor-progressing sphingosine-1-phosphate (S1P). We recently demonstrated that a specific *P. gingivalis*-derived sphingolipid, phosphoethanolamine dihydroceramide (PEDHC), suppresses human OSCC cell growth through downregulation of aCDase *in vitro*. In this study, we evaluated the potential suppressive effect of *P. gingivalis*-sphingolipids on OSCC migration and proliferation, and the relationship to host-mediated sphingolipid metabolism.
Methods: To evaluate immunohistochemistry Score (H-Score) of the aCDase, S1P, and *P. gingivalis* in human OSCC and the corresponding adjacent normal control tissue, samples were obtained from the Indiana University Melvin and Bren Simon Comprehensive Cancer Center. To examine the cell proliferation effects of *P. gingivalis*-derived sphingolipids on OSCC, the human OSCC OECM-1 cell line was incubated with various concentrations of *P. gingivalis*-derived sphingolipids for 24 h. Then, the intracellular concentration of sphingolipids and aCDase gene expression in OECM-1 cells were analyzed by liquid chromatography tandem mass spectrometry, and qPCR, respectively. We also analyzed the correlation between aCDase and both S1P and ceramide levels.
Results: The H-Score of *P. gingivalis* was lower in human OSCC tissue than the corresponding adjacent normal control tissue. The H-Score had elevated levels of aCDase and S1P at OSCC lesions compared to healthy control parts. In addition, the H-Score of aCDase had a significantly positive correlation with the H-Score of S1P. Exposure of OECM-1 cells to *P. gingivalis*-derived sphingolipid metabolites inhibited ASAH1 mRNA expression and promoted intracellular accumulation of total ceramide and dihydroceramide levels.
Conclusions: The present study demonstrated for the first time that *P. gingivalis*-derived sphingolipids have anti-proliferative effect on OSCC through the downregulation of aCDase.

Basic Science ***Faculty***

POSTER #3

DISCREPANT SENSITIVITY OF PROSTATE CANCER CELL LINES TO SUPRAPHYSIOLOGICAL ANDROGEN TREATMENT

Samuel Adams[2,3,4], Jianneng Li[1]

[1] *University of Notre Dame Department of Biological Sciences, Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Harper Cancer Research Institute, Notre Dame, IN*
[2] *University of Notre Dame Department of Biological Sciences*
[3] *Indiana University Melvin and Bren Simon Comprehensive Cancer Center*
[4] *Harper Cancer Research Institute*

Email: *sadams24@nd.edu*

Prostate cancer is the second leading cause of cancer-related deaths among men in the United States. As it is driven by androgens and the androgen receptor (AR), standard treatments include androgen deprivation therapy (ADT) and androgen receptor inhibitors (ARIs). While most cases initially respond, many eventually progress to castration-resistant prostate cancer (CRPC)—an aggressive and often lethal form of the disease. To address this challenge, next-generation therapies have been developed, including super-physiological androgen (SPA) therapy. Although counterintuitive, high-dose androgen treatment has demonstrated clinical efficacy, with up to 30% of patients responding in clinical trials. Understanding the mechanisms of SPA response and resistance is crucial for identifying predictive biomarkers and optimizing combinatorial treatment strategies. Our data indicate that SPA is effective in AR+ prostate cancer cell lines (C42, LNCaP) but has no effect in AR/GR+ (22Rv1) or GR+ (PC3) cell lines. Notably, only our GR+ cell lines exhibit resistance to SPA, suggesting that glucocorticoid receptor (GR) signaling may play a role in resistance mechanisms. We found that AR overexpression in PC3 cells causes SPA sensitivity, whereas GR overexpression in C42 cells does not diminish SPA efficacy. Further, we hypothesized that long-term GR expression or activation may be necessary for SPA resistance. However, our data show that C42 cells with sustained GR activation remain sensitive to SPA, suggesting that additional factors contribute to resistance. In the future we plan to modulate the expression levels of GR in prostate cancer cell lines to verify its effect on SPA resistance, and to investigate other steroid receptors and their role in SPA treatment.

Basic Science ***Graduate Student***

POSTER #4

ASSESSMENT OF SEX DIFFERENCES IN URETHANE-INDUCED LUNG TUMORIGENESIS IN THE "FOUR CORE GENOTYPES" MICE

Maksat Babayev[2], Carolyn Damilola Ekpruke[1], Omar Borges-Sosa[1], Dustin Rousselle[1], Chukwudike Igwe[1], Praveen Chirumamilla [1], Michelle Christine Boulos[1], Aakash Parekh[1], Matthew Louis Retzner[1], Phoenix Gray Haggard[1], Patricia Silveyra[1]

[1] *IU Bloomington School of Public Health, Department of Environmental and Occupational Health, Bloomington, IN*
[2] *IU Bloomington School of Public Health, Department of Environmental and Occupational Health*

Email: *mbabayev@iu.edu*

Lung cancer is the top cause of cancer-related deaths worldwide and remains one of the leading causes of mortality in the United States. Non-small cell lung cancer (NSCLC) represents roughly 85% of lung cancer cases, with lung adenocarcinoma (LUAD) being the most frequent subtype. Notably, sex differences have been documented in lung cancer incidence and mortality, especially among non-smokers, where LUAD is more prevalent in females. Understanding sex-specific mechanisms involved in LUAD progression is vital for the development of targeted therapies. In this project, we utilized the “four core genotypes” (FCG) model with ArnoJ (C57BL/6J) mice, in which gonadal sex is separated from the chromosomal complement. We combined this model with a urethane-induced lung cancer protocol—an established method that mirrors human LUAD—to investigate the influence of gonadal hormones and sex chromosomes on tumor progression. Six- to eight-week-old mice were administered weekly injections of urethane (1 g/kg body mass) or an equivalent volume of PBS for 10 weeks, then assessed after a 20-week tumorigenesis period. We examined cytological changes in bronchoalveolar lavage fluid (BALF), tracked body weight changes, and conducted histological analyses of fixed lung tissue. All mice receiving urethane developed neoplasias and showed elevated BALF cell counts as well as weight loss compared to controls. Among the various genotypes, XXM (female chromosomes, male gonads) displayed the most pronounced weight difference between urethane-treated and control groups. When comparing mice of the same chromosomal composition, those with XX chromosomes exhibited a greater weight change than their XY counterparts, while among mice with the same gonadal type, male gonads were associated with more significant weight loss than female gonads. In general, urethane-treated mice had higher BALF total cell counts than the PBS group, and preliminary findings suggest that mice with female gonads experienced a larger rise in BALF cell counts than those with male gonads. No notable differences emerged between XX and XY animals in this regard. Overall, these initial observations from our pilot study suggest that both sex hormones and sex chromosomes may play a role in urethane-induced weight loss and BALF changes. Ongoing analyses of histological and molecular markers will provide additional insights into sex-specific mechanisms of carcinogenesis.

Basic Science ***Graduate Student***

POSTER #5

TARGETING NUTRIENT SCAVENGING TO PROMOTE CELL DEATH IN PANCREATIC DUCTAL ADENOCARCINOMA.

Garima Baral[2], Claire Pfeffer[3], Sara Filippelli[2], Indiraa Doraivel[2], Brittany Heil [3], Brittany Allen-Petersen[1]

[1] Department of Biological Sciences & Purdue Institute for Cancer Research, Purdue University, West Lafayette, IN
[2] Department of Biological Sciences & Purdue Institute for Cancer Research, Purdue University
[3] Purdue University Interdisciplinary Sciences Program (PULSe) & Purdue Institute for Cancer Research, Purdue University

Email: *gbaral@purdue.edu*

The microenvironment of pancreatic ductal adenocarcinoma (PDAC) is comprised of >90% stroma, increasing interstitial fluid pressure and causing poor vascularization. This leads to the depletion of nutrient supply in tumor cells. To circumvent this deprivation, PDAC cells initiate macropinocytosis, an actin-driven nutrient scavenging pathway. As macropinocytosis is important for cancer cell survival under nutrient stress, inhibition of this process represents a novel strategy to suppress nutrient acquisition and drive cell death.

Protein phosphatase 2A (PP2A) is a heterotrimeric Serine/Threonine phosphatase implicated in the process of macropinocytosis. To test if PP2A contributes to macropinocytosis regulation, we used high molecular weight fluorescent Oregon Green Dextran (70 kDa) as a marker of macropinocytosis uptake as it is only internalized by macropinocytosis and no other endocytic processes. We demonstrated that pharmacological activation of PP2A by small molecule DT-061 significantly increases macropinosomes in PDAC cells. Increase in macropinocytosis is shown to promote cell survival in pancreatic cancer. However, when we treat cells with DT-061 causing increased macropinocytosis, cell viability was significantly reduced in PDAC cells. This decrease in cell viability caused by DT-061 was significantly rescued when pretreated with a Rac family inhibitor, EHT1864 that targets macropinocytosis initiation but not a pan caspase inhibitor ZVAD-FMK, suggesting that the cell death is non-apoptotic and mediated through macropinocytosis. Further, the cause of cell death through macropinosome accumulation was determined to be caused by the inability of macropinosomes to fuse with lysosomes. In conclusion, activation of PP2A mediates cell death through dysregulation of the macropinocytosis cascade, highlighting the differential regulation of macropinocytosis mediated cell survival vs. cell death.

Prevention of lysosomal fusion is known to impact nutrient reservoir in the cells. Our metabolomics study with pharmacological PP2A activation compared to control showed a depletion of key amino acids required for anabolic metabolism. This regulation by PP2A can be therapeutically leveraged to stress cells with a combination of DT-061 and amino acid transport inhibitor, V-9302. This combination drug treatment was found to be synergistic *in vitro* and significantly reduced PDAC tumor growth *in vivo*. Together, these findings indicate that activation of PP2A in late stage PDAC promotes aberrant macropinocytosis and prevents nutrient supply. These pathways can be further exploited to identify potential combination therapeutics in PDAC.

Basic Science ***Graduate Student***

POSTER #6

EXPLORING STALLED DIFFERENTIATION IN NF2-MUTANT NEURAL PROGENITORS

Noah Burket[1]

[1] *Indiana University School of Medicine, Department of Neurological Surgery*

Email: *nburket@iu.edu*

Background: *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, and surgery remains the standard of care for 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.

Methods: We performed *NF2* CRISPR knockouts in neuroepithelial 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: Our preliminary results show that these *NF2*-mutant NES cells do not undergo normal differentiation, as they continue to express neural progenitor genes and retain a neural progenitor morphology in vitro. They also form clusters in vitro which continue to proliferate and form clusters when split into a new dish. These *NF2*-mutant cells appear to have proliferative potential following differentiation, whereas wildtype cells primarily form neurons and other mature glial cells.

Discussion: Our data supports that intact *NF2* may be required for normal neural progenitor differentiation, and that mutations of this gene prevent these cells from giving rise to mature neurons and glia. Given the known tumorigenic effects of *NF2* loss, this model may provide insights into early SP-EPN development, as well as serve as platform to identify novel therapies for this tumor.

Basic Science ***Graduate Student***

POSTER #7

UNDERSTANDING THE INTERACTION BETWEEN HSF1 AND ERRα IN TRIPLE NEGATIVE BREAST CANCER

Sunandan Chakrabarti[5], Yuan Feng[1], David Adelfinsky[2], Jason Tennessen[3], Richard Carpenter[4]

[1] Department of Biology, Indiana University-Bloomington, Bloomington, IN 47405, Bloomington, IN, Bloomington , IN
[2] Medical Sciences, Indiana University School of Medicine-Bloomington, Bloomington, IN 47405, Bloomington, IN, Bloomington, IN
[3] Department of Biology, Indiana University-Bloomington, Bloomington, IN 47405, Bloomington, IN, Bloomington, IN
[4] Medical Sciences, Indiana University School of Medicine-Bloomington, Bloomington, IN 47405, Bloomington, IN, Biochemistry and Molecular Biology, Indiana University School of Medicine-Bloomington, Bloomington, IN 47405, Bloomington, IN, Bloomington, IN
[5] Medical Sciences, Indiana University School of Medicine-Bloomington, Bloomington, IN 47405, Bloomington, IN

Email: *sunchakr@iu.edu*

Breast Cancer is the most common type of cancer in women with 1 in 8 women at risk for developing 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, is essential for oncogenesis and has pleiotropic roles in tumors including proteostasis, epithelial-to-mesenchymal transition (EMT), and suppression of immune-mediating killing among many others. 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 assessed how they influence the activity of the other and observed that ERRα enhanced HSF1 activity while expression of HSF1 alone induced ERRα activity. 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. 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. Given that HSF1 and ERRα regulate stress response and metabolic homeostasis in cells, future studies will investigate how this complex contributes to 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 #8

INVESTIGATING THE ROLE OF ENTEROENDOCRINE CELLS IN THE INITIATION OF MIGRATION IN BRAF-MUTATED COLORECTAL CANCER

Averi Chakraborty[1], Heather M. O'Hagan[2]

[1] Cell, Molecular and Cancer Biology Graduate Program, Indiana University School of Medicine, Bloomington, IN, 47405, Bloomington, IN
[2] Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN, 47405, USA, Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN, 46202, USA, Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, 462

Email: *avchakra@iu.edu*

Colorectal cancer (CRC) is recognized as the second leading cause of death worldwide. Despite advancements in screening methods like colonoscopy and sigmoidoscopy, CRC often remains undetected until advanced stages. Approximately 10% of all CRC cases contain activating BRAF mutations (most commonly $BRAF^{V600E}$), which are linked to poor patient prognosis, treatment resistance, and metastasis. However, the specific identity and mechanism of the migration-initiating cells within the primary $BRAF^{V600E}$ mutant CRC are still unknown. Recent studies have highlighted the presence of neuroendocrine cells in multiple aggressive and therapy-resistant cancers, including prostate, lung, pancreas, and ovarian cancers. In the normal colon epithelium, neuroendocrine cells exist as specialized hormone-secreting cells known as enteroendocrine cells (EECs). Our lab has previously shown an enrichment of EEC progenitors in BRAF-mutant CRC. Through additional *in vivo* experiments, we have also observed beta-3-tubulin (TUBB3), a marker for EECs, in cells escaping the epithelial boundary of more advanced BRAF-mutant colorectal tumors. Based on this observation, we hypothesized that these intestinal neuroendocrine cells (EECs) contribute to the initiation of migration in BRAF-mutant CRC. Our preliminary results suggest that increased EEC differentiation correlates with upregulation of epithelial to mesenchymal transition (EMT)–related markers (N-cadherin, Vimentin, and Snail) in mouse $BRAF^{V600E}$-mutant tumor-derived organoids. Additionally, we found that partially knocking out a key transcription factor essential for EEC lineage differentiation, Neurogenin-3, resulted in decreased expression of the EMT-related markers. These preliminary findings bolster our hypothesis that EECs play a role in initiating migration in BRAF-mutant CRC. We aim to investigate two primary questions: Are EECs crucial for the initiation of migration, and through what mechanism do they promote EMT? Given their predominant secretory nature, EECs could promote EMT in surrounding tumor epithelial cells through secreted factors. However, EECs can also alter their phenotypes to form cellular extensions (neuropods) to facilitate neural communication and could therefore adopt a mesenchymal phenotype to initiate migration in cancer. Currently, we are developing a method to capture and observe the migrated organoids without disrupting the surrounding matrix. Through immunocytochemistry, we will identify the specific location of EECs in the migrating organoids to determine if they are present at the migration front. We are also developing methods to eliminate the EEC lineage from the organoids to better understand their role in promoting migration in $BRAF^{V600E}$-mutant CRC. Our study will clarify the role of EECs in CRC migration and provide mechanistic insights to help restrict their involvement. The findings can lead to clinical strategies aimed at inhibiting EEC involvement to improve patient prognosis and mitigate the progression of CRC.

Basic Science ***Graduate Student***

POSTER #9

THE ROLE OF GENERAL CONTROL NONDEREPRESSIBLE 2 (GCN2) KINASE IN LEUKEMOGENESIS AND THERAPEUTIC RESPONSE TO L-ASPARAGINASE

Rodney Claude[1]

[1] *Biochemistry and Molecular Biology*

Email: *rclaude@iu.edu*

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer, affecting the lymphoid cells of both B and T lineages. Most ALL cells are auxotrophic for asparagine, a nonessential amino acid for protein synthesis, due to the low expression of asparagine synthetase (ASNS), a rate-limiting enzyme for de novo biosynthesis of asparagine. Standard ALL treatment takes advantage of this vulnerability by giving patients L-asparaginase, a bacterial enzyme that depletes the circulating asparagine. However, previous work from our lab and others has shown that some ALL cells become resistant to L-asparaginase treatment through the induction of ASNS expression. Mechanistically, amino acid starvation activates the general control of nonderepressible 2 (GCN2) kinase, leading to the accumulation of the ATF4 transcriptional factor. ATF4, in turn, is recruited to the promoter of the ASNS gene to activate its transcription. However, the role of GCN2 kinase in the process of leukemogenesis under nutrient limiting environment has not been established. Here, we found that, in a mouse T-ALL model driven by a KRas(G12D) mutation, ATF4 and ASNS expression were strongly elevated in leukemic tissues. However, germline deletion of the Gcn2 did not delay leukemogenesis in primary T-ALL and secondary T-ALL generated by transplanting the pre-leukemic bone marrow cells into Gcn2 wildtype recipient mice. Although GCN2 inactivation does not affect leukemogenesis, it sensitizes T-ALL cells to Lasparaginase treatment in vitro and in vivo. In addition, a small molecule inhibitor of GCN2, GCN2iB, synergistically inhibits leukemic growth and survival in the mouse T-ALL model and human T-ALL lines where the basal expression of ASNS is low. Finally, we tested a newly developed inhibitor of ASNS (ASX-173), which selectively killed leukemic cells in the absence of exogenous asparagine even though their basal level expression of ASNS is high. Taken together, our results suggest that targeting GCN2 or ASNS will have therapeutic implications in ALL patients who become resistant to L-asparaginase treatment.

Basic Science ***Graduate Student***

POSTER #10

DEFINING THE MECHANISMS OF CANCER SPECIFIC TRANSCRIPTION FACTOR, TGLI1

Haddie DeHart[1], Nolan Gregg[2], Julie Heldman[2], Richard Carpenter[1]

[1] *Cell, Molecular, and Cancer Biology; IU School of Medicine-Bloomington*
[2] *Indiana University*

Email: *hkdehart@iu.edu*

Glioblastoma (GBM) is the most common malignant central nervous system tumor in adults, with a five-year survival rate below 7%. Truncated GLI1 (tGLI1), an alternative splicing isoform of GLI1, is a cancer-specific variant highly expressed in GBM but absent in normal tissue. Despite losing 41 amino acids, tGLI1 retains all functional domains of GLI1 and responds to sonic hedgehog signaling similarly. However, tGLI1 functions as a gain-of-function transcription factor, activating unique genes that promote invasion, migration, angiogenesis, and stemness. To explore tGLI1's DNA binding pattern, we conducted ChIP-sequencing (ChIP-seq) and found only 14% overlap between GLI1 and tGLI1 binding sites, indicating distinct genome-wide interactions. To investigate whether protein interactions contribute to this difference, we performed immunoprecipitation (IP) with mass spectrometry (mass spec), identifying 45 GLI1- and 52 tGLI1-associated proteins, with only 29% overlap. Gene ontology analysis revealed 43% of tGLI1-specific proteins are linked to RNA processes. Among them, NONO, an RNA- and DNA-binding protein, interacted with tGLI1,enhanced its nuclear localization, and altered the expression of tGLI1 and its targets. These findings suggest NONO may mediate tGLI1-driven phenotypes in GBM, warranting further investigation into its role in GBM progression.

Basic Science ***Graduate Student***

POSTER #11

ONCOGENIC ETS TRANSCRIPTION FACTORS IN PROSTATE CANCER AND EWING SARCOMA ARE INTERCHANGEABLE WHEN AVOIDING REPRESSION BY A PRC2/FOXO1 COMPLEX.

Nicholas Downing[1], Katelyn Mills[1], Peter Hollenhorst[1]

[1] *Medical Sciences, Indiana University, Bloomington*

Email: *nfdownin@iu.edu*

Genes encoding ETS family transcription factors are altered by chromosomal rearrangements in 60-70% of prostate cancers and nearly all Ewing sarcomas. Ewing sarcoma rearrangements result in chimeric fusion of ETS proteins to the RNA-binding protein EWS. Prostate cancer rearrangements reasult in aberrant expression of ETS proteins such as ETV1, ETV4, ETV5 or ERG that can interact with wild-type EWS, suggesting common mechanisms between these diseases. Here, we find that ETV1, ETV4, and ETV5 can phenocopy EWS/FLI1 in Ewing sarcoma cell lines. However, rescue of EWS/FLI1 knockdown by ERG requires an ERG mutant that disrupts its interaction with PRC2. This suggests that EWS/ERG fusion breakpoints are selected in Ewing sarcoma to avoid PRC2 interactions. We identify an endogenous PRC2/FOXO1 complex and demonstrate that FOXO1 bridges the ERG/PRC2 interaction. AKT-mediated degradation of nuclear FOXO1 and subsequent loss of the ERG/PRC2 interaction provides a mechanism for ERG synergy with PTEN deletion in prostate cancer.

Basic Science ***Graduate Student***

POSTER #12

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

Savannah Epstein[1]

[1] *Anatomy, Cell Biology, and Physiology*

Email: *savepste@iu.edu*

Cancer cachexia is a multifactorial wasting syndrome which entails muscle mass loss with or without the loss of adipose tissues. Pancreatic cancer, the 3rd leading cause of cancer associated deaths, has the highest incidence of cancer cachexia at 80%. 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, hypothesizing that downstream targets may be more precise targets for potential therapeutic intervention. Krüppel-like factor 10 (KLF10) is a known downstream target of TGF-β which has been proven to affect the metabolism and development of muscle.

In this study we utilized *in vitro*, *in vivo,* and genetic tools/modelsto define the role of KLF10 in pancreatic cancer associated muscle wasting. KLF10 expression was also queried in the muscle from patients with cancer cachexia.

KLF10 expression is increased in all in *vitro*, *in vivo*, and human data sets. Our work shows 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 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 might be an intriguing protein to study in 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 #13

LYSINE METHYLATION-MEDIATED MODULATION OF 14-3-3 BINDING: TOWARD UNDERSTANDING DYSREGULATED SIGNALING IN CANCER

Taylor Evans[4], Lawrence Quilliam[1], Emma Doud[2], Evan Cornett[3]

[1] *Department of Biochemistry and Molecular Biology, IU Simon Comprehensive Cancer Center, Indianapolis, IN*
[2] *Department of Biochemistry and Molecular Biology, IU Simon Comprehensive Cancer Center, IU Center for Proteome Analysis, Indianapolis, IN*
[3] *Department of Biochemistry and Molecular Biology, IU Simon Comprehensive Cancer Center, IU Center for Computational Biology and Bioinformatics, Stark Neurosciences Research Institute, Indianapolis, IN*
[4] *Department of Biochemistry and Molecular Biology*

Email: *evanstay@iu.edu*

In recent years, aberrant lysine methylation has emerged as a key driver in cancer signaling, but the functional consequences of this post-translational modification on non-histone proteins remain poorly understood. One such protein family is the 14-3-3 family, a group of highly conserved regulatory proteins that play central roles in cell cycle control, apoptosis, and cellular stress response, all of which are processes frequently dysregulated in cancers. Recent proteomic studies by our group have identified lysine methylation on multiple 14-3-3 isoforms, but the biological significance of these modifications remain unexplored. This project aims to define how lysine methylation at a conserved site within the 14-3-3 binding pocket affects protein-protein interactions, with a broader goal of understanding how it modulates signaling cancer-relevant signaling pathways. To investigate this, a multidisciplinary strategy combining chemical biology, proteomics, and molecular cell biology are being employed. For *in vitro* studies, methyl-lysine analog chemistry is being used to install defined methylation states (mono-, di-, and trimethylation) on the key lysine residue within 14-3-3. This approach allows for direct assessment of how various methyl-lysine states impact binding to known binding partners by way of *in vitro* binding assays; it also allows for exploring proteome-wide changes to client interactions through immunoprecipitation-mass spectrometry. Thus far, our results indicate methylation of 14-3-3 significantly reduces its binding to a phosphorylated binding partner. As a complement to this aspect of the study, a proteomics-based strategy for identifying the lysine methyltransferase(s) (KMTs) responsible for modifying 14-3-3s in cells is being developed. This involves a systematic KMT overexpression screen combined with global proteomics. Identifying the responsible KMT will enable downstream studies of its function in cancer contexts where 14-3-3 activity is altered. Given the well-established roles of 14-3-3 proteins in multiple cancer types, where altered client interactions contribute to proliferation and resistance to therapy, understanding how lysine methylation tunes these interactions could reveal novel mechanisms of dysregulation. Furthermore, several KMTs are already being investigated as therapeutic targets in several cancer types, suggesting translational potential. Together, this work provides a framework for investigating how site-specific lysine methylation regulates protein networks and may contribute to oncogenic signaling. By focusing on a central signaling hub protein like 14-3-3, this research will shed light on an underexplored aspect of cancer-relevant regulation.

Basic Science ***Graduate Student***

POSTER #14

UNDERSTANDING THE MECHANISM OF PP2A ACTIVATION ON MACROPINOCYTOSIS

Sara Filippelli[2], Garima Baral[2], Indiraa Doraivel[2], Brittany Allen-Petersen[1]

[1] *Department of Biological Sciences, Purdue University, West Lafayette, IN*
[2] *Department of Biological Sciences, Purdue University*

Email: *sfilippe@purdue.edu*

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer with limited treatment options. The tumor microenvironment of PDAC is nutrient deplete, leading cancer cells to activate alternative pathways, such as macropinocytosis, to acquire nutrients needed to support survival. During macropinocytosis, extracellular fluid isinternalized into intracellular vesicles called macropinosomes which fuse with lysosome to replenish nutrients.Previous work has shown that the tumor suppressor protein phosphatase 2A (PP2A) is involved in the regulation of macropinocytosis. PP2A is a heterotrimeric complex that is made up of a core complex consisting of the "A" scaffolding subunitand "C" catalytic subunit which will then associate with one of 16"B" regulatory subunits, which determines substrate specificity.However, the individual contribution of B subunits to macropinocytosis is not well understood. To determine the impact of PP2A activation on macropinocytosis, we treated PDAC cells with DT061, a small molecule activator of PP2A. DT061 treatment increasedmacropinosome accumulation and decreasedmacropinosome-lysosome fusion.To investigatethe contribution of individual B subunits to macropinocytosis, stable overexpression (OE)of B56 and B55 family memberswere generated. B56α wasthe only subunitto significantly increase macropinosome accumulation compared to controls.Using Co-immunoprecipitation, we identifiedthat B56a interactswitha critical lipid kinase implicated in lysosomal fusion. Future studies willinterrogate this impact of this interaction on macropinocytosisand determinethe functional consequence of PP2A-regulated phospho-sites.Together, these findingsidentify novel posttranslational mechanisms that contribute to PDAC macropinocytosis and implicate PP2A as a novel therapeutic target to suppress macropinocytosis-driven nutrient scavenging.

Basic Science ***Graduate Student***

POSTER #15

EXPLOITING GCN2-DEPENDENT VULNERABILITIES IN ASPARAGINE BIOSYNTHESIS FOR THE TREATMENT OF ADVANCED PROSTATE CANCER

Madison Gerbig[1,2]

[1] *Department of Biochemistry and Molecular Biology, Indiana University School of Medicine*
[2] *IU Simon Comprehensive Cancer Center, Indiana University School of Medicine*

Email: *mgerbig@iu.edu*

Prostate cancer (PCa) is the second leading cause of cancer related deaths in men, with a poor overall 5-year survival rate of 30% in advanced late-stage disease. Androgen receptor (AR) signaling is a central driver of PCa, and androgen deprivation therapies are the standard of care for early-stage tumors. However, resistance to these treatments is a major driving force behind the advancement to metastatic, AR-resistant disease, emphasizing the importance for more targeted therapies for prostate cancer. Our laboratory discovered that PCa cells rely on a stress sensory protein kinase known as general control nonderepressible 2 (GCN2) to import and synthesize amino acids that are required to promote cell proliferation. GCN2 is a key enzyme in the integrated stress response (ISR), a pathway which directs translation and transcription to mitigate cell damage in response to diverse intracellular and extracellular stressors. As a result of increased demands for cell proliferation and harsh microenvironments, PCa cells can rely on GCN2 for nutrient repletion strategies to promote tumor progression. While depletion of GCN2 lowers amino acid levels and blocks growth of PCa in *in vitro* and *in vivo* models, loss of GCN2 does not induce cell death. A CRISPR interference screen was conducted by our lab to identify genes whose reduced expression showed synthetic lethality *in vivo* after the loss of GCN2 function, which suggested that targeting genes in certain metabolic processes, including amino acid biosynthesis, could be effective at killing PCa in combination therapies with GCN2.Collectively, we hypothesize that targeting GCN2 in combination with the asparagine biosynthesis enzyme, asparagine synthetase (ASNS), would be an effective strategy for starving and limiting growth of PCa cells. Methods of starving cancer cells for asparagine have notably been effective in the treatment of acute lymphoblastic leukemia, due to low expression of ASNS, which converts L-aspartate to L-asparagine using L-glutamine as a nitrogen source. The expression of ASNS in PCa cells is inducible by GCN2 and the ISR, but efforts in targeting ASNS have been limited.However, a recently reported inhibitor of ASNS, designated ASX-173, may provide a mechanism for depleting asparagine in PCa, which we believe would be further exacerbated with loss of GCN2. In this project, we aim to test the effects of targeting GCN2 and ASNS together in prostate cancer cell lines, organoids, and *in vivo* models, with the objective to uncover novel therapeutic methods for the treatment of AR-resistant prostate cancer.

Basic Science ***Graduate Student***

POSTER #16

TETV1 SWITCHES FROM A REPRESSOR TO AN ONCOGENIC ACTIVATOR IN A RAS/MAPK-DEPENDENT MANNER

Ethan Golditch[2], Peter Hollenhorst[1]

[1] *Department of Biochemistry and Molecular Biology, Indiana University School of Medicine , Bloomington, IN*
[2] *Department of Biology, Indiana University Bloomington*

Email: *egoldit@iu.edu*

In the majority (~67%) of prostate cancers, an ETS transcription factor is aberrantly overexpressed in the prostate due to chromosomal translocation. Of these, the most frequently implicated is ERG (50%), followed by ETV1 (5-10%), ETV4 (2-5%), and ETV5 (~1%). Previous studies in the lab and others have shown that expression of ERG in the prostate induces prostatic intraepithelial neoplasia (PIN) and epithelial-mesenchymal transition (EMT). ERG has also been shown to synergize with the PI3K/AKT and Ras/MAPK to initiate tumorigenesis, and with the TLR4 pathway to drive migration. However, less is known regarding which biomolecular pathways are utilized by ETV1 to drive prostate cancer. In my studies I have found that a clinically relevant truncated ETV1 (tETV1) can cooperate with the Ras/MAPK pathways in a context-dependent manner. Specifically, while tETV1 paradoxically represses EMT in the normal immortalized prostate cell line RWPE-1, KRAS overexpression enables induction of EMT. Interestingly, in both instances, tETV1 drives transwell migration, suggesting that Ras/MAPK signaling is not essential for tETV1 to induce cellular migration. I have also found that the mutation of MAPK and MAPKAPK phosphorylation sites to alanine (S/T ◊ A) fails to reduce migration and can increase the ability of tETV1-positive RWPE-1 cells to migrate. Additionally, while tETV1 expression leads to a loss of colony formation and basal epithelial markers, expression of phosphonull mutants of tETV1 rescues colony formation and drives basal differentiation. Aside from changes in oncogenic phenotypes, tETV1 can also differentially regulate the Ras/MAPK and PI3K/AKT pathways. While overexpression of tETV1 leads to an increase in both pathways in the normal prostate cell line, PNT2, its expression represses both in RWPE-1. Therefore, while the oncogenic potential of tETV1 is dependent on its background, it remains to be elucidated which factors precipitate this change. It also remains unclear which direct targets of tETV1 are required for participation or exclusion from these regulatory mechanisms. Better characterization of these changes will enable the development of therapies which simultaneously keep tETV1 in a repressive state and disrupt the Ras/MAPK and PI3K/AKT signaling axes.

Basic Science ***Graduate Student***

POSTER #17

PHYSIOLOGIC OXYGEN EXPANSION OF HEMATOPOIETIC STEM AND PROGENITOR CELLS (HSPCS) ENHANCES LYMPHOCYTE RECOVERY FOLLOWING TRANSPLANTATION

Sarah Gutch[4], James Ropa[1], Lindsay Wathen[1], Gracie Whitacre [1], Wendy Deras[1], Wouter Van't Hof[2], Maegan Capitano[3]

[1] *Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis , IN*
[2] *Cleveland Cord Blood Center, Cleveland , OH*
[3] *Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN*
[4] *Department of Microbiology and Immunology, Indiana University School of Medicine*

Email: *ssgutch@iu.edu*

Rapid recovery of lymphocytes post hematopoietic cell transplantation (HCT) is correlated with positive patient outcome in treatments of hematologic disease. Few effective clinical therapies exist to enhance lymphocyte recovery, indicating a clear unmet need. *Ex vivo* expansion of umbilical cord blood (CB) HSPCs is an FDA approved therapy to increase recovery of critical hematopoietic cells such as neutrophils and platelets, but impact on lymphocytes remains uncertain. Expansion under physiological oxygen (1-14% O_2) results in increased lymphoid-biased RNA levels and lymphocyte progenitor cell numbers. After *ex vivo* expansion of murine HSPCs, nucleated cell counts increase and frequencies of HSPCs decrease as O_2 tension increases. As a ratio of injected cells, mice transplanted with HSPCs expanded at 1% O_2 had increased natural killer cell frequencies at weeks 1 and 10 and increased CD4 and dendritic cell frequencies at week 5 post HCT in the peripheral blood (PB) compared to transplantation with ambient air expanded (21% O_2) HSPCs. Transplantation with huCD34+ CB HSPCs expanded at 1-14% O_2 results in increased CD3+/CD19+ frequencies in the PB at week 10 post HCT compared to ambient air expansion. Transcriptomic analysis reveals differential mitochondrial gene expression in lymphocyte progenitors expanded under 1% O_2 compared to 21%O_2. Our results suggest expansion under physiological O_2 is a viable strategy for improving lymphocyte recovery and patient outcome post HCT.

Basic Science ***Graduate Student***

POSTER #18

Remove

THE RXR AGONIST MSU-42011 AND THE MEK INHIBITOR SELUMETINIB REDUCE TUMOR BURDEN BY DECREASING PERK LEVELS AND MODULATING IMMUNE CELL POPULATIONS WITHIN THE NF1 TUMOR MICROENVIRONMENT.

Pei-Yu Hung[1], Jessica A. Moerland[2], Karen T Liby[3]

[1] *Department of Physiology, Michigan State University, East Lansing, MI*
[2] *Department of Pediatrics and Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN*
[3] *Department of Medicine, Division of Hematology/Oncology, Indiana University School of Medicine, Indianapolis, IN*

Email: *hungpei@iu.edu*

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 the drug has 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 is 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 and mouse MPNST cells, treatment with 200 nM MSU-40211 or 50 nM selumetinib for 3 hours reduced pERK protein levels compared to 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 the combination treatment. The combination of MSU42011 and selumetinib also significantly reduced tumor growth and reduced pERK levels and tumor-promoting CD206+ macrophages in both a LL2 model of lung cancer driven by an activating *Kras* mutation and a syngeneic mouse model of MPNST. 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 genetically engineered model of PNF and MPNST.

Basic Science ***Graduate Student***

POSTER #19

ELUCIDATING RPA'S ROLE IN ALTERNATIVE DNA REPAIR PATHWAYS AND ITS IMPACT ON GENOMIC INSTABILITY IN OVARIAN CANCER

Jessica Kersey[1,2], John Turchi[1,2,3,4]

[1] *Department of Biochemistry and Molecular Biology, Indiana University School of Medicine*
[2] *IU Simon Comprehensive Cancer Center*
[3] *Tom and Julie Wood Family Foundation*
[4] *Department of Medicine, Indiana University School of Medicine*

Email: *kerseyj@iu.edu*

Ovarian cancer is characterized by excessive genomic instability due to high mutational rates and chromosomal aberrations, which drive treatment resistance and recurrence.Despite therapeutic advancements, survival trends for ovarian cancer have seen only marginal improvements when compared to other common cancers. DNA double-stranded breaks (DSBs) drive genomic instability. DSBs are repaired through 4 pathways: non-homologous end joining (NHEJ), homologous recombination (HR), microhomology-mediated end joining (MMEJ), and single-strand annealing (SSA). Defective repair pathways such as HR, which occurs in ~50% of ovarian cancer cases, lead to increased reliance on alternative, error-prone pathways like MMEJ and SSA. Although the complete mechanism and the full spectrum of repair proteins involved in MMEJ and SSA remain unknown, the proposed mechanisms of microhomology-mediated end joining (MMEJ) and single-strand annealing (SSA) involve resection of the 5' DNA strand to expose regions of homology required for pathway completion. Notably, the role of RPA, the major single-strand DNA bring protein, has yet to be studied within the context of these pathways, both of which have the potential to involve extensive regions of ssDNA. The RPA ssDNA binding inhibitor, NERx329, was used to determine RPA's role in DSB repair pathways MMEJ and SSA. The impact of the inhibitor was measured by assessing the repair of pathway specific, extrachromosomal reporter plasmids for each pathway independently. A luminescence assay using Firefly (transfection control) and NanoLuc (pathway repair reporter) luciferase reporter genes was used to measure the extent of repair in human epithelial ovarian cancer cell line SKOV3, and the human embryonic kidney cell line HEK293. We find that the resection-independent MMEJ is dependent on RPA activity in SKOV3 cells, while MMEJ in HEK293 cells was unaffected by the presence of NERx329. Interestingly, for resection-dependent MMEJ response to the positive inhibition control, ART558, a POLQ inhibitor, was much lower in HEK293 cells and not significant in SKOV3 cells. SSA experiments with H1299 Cas9 control and XPF KO cell lines show that SSA is dependent on XPF. Additional experiments using XPF inhibitor, 16168, at increasing concentrations in the H1299 Cas9 cell line showed no change in SSA activity indicating this assay may not be optimal for use of all inhibitors. Similarly, reporter constructs for HR showed no impact from inhibition of RPA or RAD51, via NERx329 and RI-1, both of which are proteins known to be implicated in this pathway. Collectively, these data show the complexity of DNA repair mechanisms and how cells make decisions on which one to carry out. Future experiments will include extrachromosomal assays that generate a pathway specific repair scar which can be sequenced to analyze extent of repair as well as chromosomal reporter assays to complement these results.

Basic Science ***Graduate Student***

POSTER #20

CHIRAL-ASSISTED SIRNA LOADING AND PH-RESPONSIVE SMALL EXTRACELLULAR VESICLES FOR HIGHLY EFFICIENT GENE DELIVERY IN BREAST CANCER

Gaeun Kim[2], Runyao Zhu[1], Sihan Yu[1], Bowen Fan[1], Hyunsu Jeon[1], Jennifer Leon[1], Matthew J. Webber[1], Yichun Wang*[1]

[1] *Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, IN*
[2] *Department of Chemical and Biomolecular Engineering, University of Notre Dame*

Email: *gkim4@nd.edu*

Small extracellular vesicles (sEVs) carry biomolecules sorted from their cell-of-origin and transport this diverse cargo for cell-to-cell communication. This intercellular interaction, along with their structural and physiological stability, highlights sEVs as promising nanocarriers for drug delivery. Although advances have recently been made in sEV-based drug delivery platforms, challenges from insufficient targeting and low therapeutic efficacy continue to hinder their practical clinical progress. Recent studies reported that sEV uptake depends on their cell origins and shows preferential uptake by parental cells, known as the homing effect, which presents an opportunity to utilize this feature to enhance its targeting ability. However, this enhanced uptake occurs through specific ligand-receptor interactions that facilitate endocytosis but only about 24% of endocytosed sEVs escape lysosomes and avoid undesirable degradation. This highlights the need for strategies to improve lysosomal escape to fully harness their homing effect for targeted drug delivery. Moreover, despite extensive exploration of drug loading methods, challenges such as lipid deterioration, protein denaturation, and low loading efficiency (<20%) contribute to the suboptimal efficacy of sEVs. In this study, we utilized chiral graphene quantum dots (GQDs) for siRNA loading into sEV via nanoscale chiral interactions, achieving > 60% loading efficiency while preserving sEV structural integrity. Additionally, we synthesized a cholesterol-conjugated pH-responsive peptide to functionalize sEV membranes through hydrophobic interactions, facilitating lysosomal escape in response to pH changes within the lysosome. We finely optimized the integration of these two non-aggressive and passive approaches, which, in turn, preserved the inherent bioactivity of breast cancer cell-derived sEVs to leverage the homing effect for targeted therapy. The GALA peptide decorated on sEVs facilitated lysosomal escape and enabled cytoplasmic cargo delivery through charge conversion, promoting fusion with the lysosomal membrane. This resulted in a 1.74-fold increase in cytoplasmic cargo delivery and achieved ~72% gene knockdown along with high-efficiency siRNA loading. To address chemotherapeutic resistance in breast cancer associated with a stiff extracellular matrix (ECM), we successfully delivered siTGF-β RNA intracellularly, resulting in reduced ECM stiffness and enhanced drug sensitivity. This was further demonstrated by a combination treatment resulting in low cell viability after two days in the cytotoxicity test, along with a notable inhibition of colony formation in the one-week clonogenic assay. Overall, our approaches have significant potential to enhance sEV-based gene delivery for breast cancer and accelerate clinical translation.

Basic Science ***Graduate Student***

POSTER #21

THE ROLE OF THE PARASPECKLE, A PHASE SEPARATED NUCLEAR BODY, IN EWS ACTIVATED CANCERS

Renee Kinne[1], Peter Hollenhorst[1]

[1] *School of Medicine Bloomington*

Email: *rekinne@iu.edu*

The ETS family of transcription factors become aberrantly activated in multiple cancers. This activation is dependent on the co-activator EWS in both prostate cancer and Ewing sarcoma, an adolescent bone cancer. In prostate cancer, EWS is a binding partner necessary for oncogenic ETS, primarily ERG, activation. 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 liquid-liquid phase separation (LLPS) and acts as a transcriptional activation domain. In cells, EWS is found in phase separated bodies called paraspeckles. Paraspeckles are nuclear bodies that have a role in transcriptional regulation, but their role in cancer is still not understood. There has also been evidence that NEAT1, the essential RNA scaffolding of the paraspeckle, has a role in tumor progression in prostate cancer and is linked with poor prognosis. Therefore, we hypothesize the paraspeckle may be a mechanism of transcriptional regulation for EWS activated cancers.

I have shown that both ERG and EWS/FLI1 interact with the paraspeckle through NEAT1 RIP. Prostate cancer and Ewing sarcoma lines showed paraspeckle presence through NEAT1 RNA-FISH. Knock down (KD) of two necessary paraspeckle components, NEAT1 or FUS, in Ewing sarcoma cells reduce paraspeckle presence, colony growth, and EWS/FLI1 activity at the transcriptional level. KD of FUS and NEAT1 also increased migration, which is consistent with EWS/FLI1 loss. I have shown that ERG colocalizes with EWS granules in a purified protein droplet assay. This suggests that oncogenic ETS can participate in EWS LLPS even when not fused like in EWS/FLI1. Taken together, these data suggest that the paraspeckle has a role in EWS/FLI1 activity in Ewing Sarcoma and that components of the paraspeckle interact with ERG. Because ERG and FLI1 are close relatives, sharing around 70% homology, there is potential that the paraspeckle acts as a mechanism of oncogenic transcription in prostate cancer as well as Ewing sarcoma.

Basic Science ***Graduate Student***

POSTER #22

MAST CELLS INTERACT DIRECTLY WITH COLORECTAL CANCER CELLS TO PROMOTE EPITHELIAL-TO-MESENCHYMAL TRANSITION

Rosie Lanzloth[1]

[1] *Genome, Cell and Developmental Biology Graduate Program, Department of Biology, Indiana University, Bloomington, IN, 47405*

Email: *rlanzlot@iu.edu*

Colorectal cancer (CRC) represents the second most common cause of cancer-related deaths in men and women combined in the US. Mucinous CRC is a subtype of CRC characterized by the production of mucin, which is associated with resistance to therapies and lower overall survival. Our long-term goal is to find better therapeutic strategies to treat mucinous CRC. Our objective is to understand what contributes to the aggressiveness of mucinous CRC. Our laboratory demonstrated that mast cells (MCs), a type of granulocytic immune cell, are enriched in mucinous 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. My results demonstrate that epithelial-to-mesenchymal transition (EMT) is induced in mucinous CRC cells upon direct interaction with MCs. The expression of EMT-related markers, such as Slug, ZEB2, and vimentin increase at the gene and/or protein level in CRC cells when they are cocultured with MCs. The migratory ability of mucinous CRC cells also increases after direct interaction with MCs. Additional data revealed that MCs promote EMT-related marker expression in CRC cells in an integrin-mediated, and calcium- and contact-dependent fashion. Lastly, my results demonstrate that MC-encoded molecules, such as vimentin mRNA, are directly transferred from MCs to CRC cells. These results suggest that targeting MC-CRC interactions, particularly through modulating integrin pathways, could offer new therapeutic strategies for aggressive CRC subtypes.

Basic Science ***Graduate Student***

POSTER #23

MIR MIR ON THE WALL, WHO'S THE MOST TUMOR-SUPPRESSIVE OF THEM ALL?

Basilia Liu[4], Ashlie Beuter[4], Sagar Utturkar Utturkar[5], Nimod Janson[1], Anthony Murphy[2], Nadia Lanman[3], Jason Hanna[1]

[1] *Biological Science Program, Purdue University, West Lafayette, IN*
[2] *Biological Science Program, Purdue University, West Lafayette, IN*
[3] *Department of Comparative Pathobiology, Purdue University, West Lafayette, IN*
[4] *Biological Science Program, Purdue University*
[5] *Department of Comparative Pathobiology, Purdue University*

Email: *liu3337@purdue.edu*

Angiosarcoma (AS) is an aggressive tumor resulting in a very poor prognosis for patients. MicroRNAs (miRNAs) regulate gene expression and play important roles in a variety of diseases including cancer. Our previous studies demonstrate miRNA loss leads to AS in mice. To further understand the role of miRNAs that function as critical tumor suppressors in AS, we performed a miRNA-focused CRIPSR-Cas9 screen. The gRNA library was transduced into a human AS cell line expressing doxycycline (dox)-inducible Cas9. After passaging the cells for 28 days, sgRNA amplicon sequencing was performed to determine the change in the frequency of gRNAs. We anticipated that the loss of function of tumor suppressing miRNAs would lead to gRNA enrichment in the cells. Indeed, three miRNAs were identified as hits with significant enrichment of multiple gRNAs, including miR-200b, miR-181b, and miR-410. We hypothesize these miRNAs act as tumor suppressors and reduce proliferation and cell viability in AS. Based on our RNA-seq data, all three miRNAs are expressed in normal endothelial cells. Additionally, miR-410, miR-181b and a miR-200 family member are significantly downregulated in a human AS cell line and miR-410 is frequently deleted in AS patient samples. We are currently conducting functional validation studies by overexpressing these miRNAs in a panel of AS cell lines by lentiviral based expressing the pre-miRNAs. In preliminary results, the overexpression of miR-410 consistently inhibits cell proliferation and colony formation ability in both human and mouse AS cell lines. Additionally, to gain mechanistic insights for these miRNAs, a novel technique called AgoTRIBE was used to identify important mRNA targets regulated by miRNAs. In AgoTRIBE, the miRNA effector Ago2 is fused to ADAR's RNA editing domain, directing ADAR activity to miRNA natural targets. 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 AS cell line. In preliminary data, we validated the protein expression of ADAR and ADAR-Ago upon dox treatment and miRNA mediated target downregulation in cells expressing AgoTRIBE through dual luciferase assay. This project represents the first global screen on miRNAs in AS. Functional validation of the hits from the screen in combination with the mechanistic insights from AgoTRIBE can provide fundamental evidence for the role of tumor suppressive miRNAs in AS.

Basic Science ***Graduate Student***

POSTER #24

EXPLORING THE BALANCE OF SETD8 AND PHF8 CATALYTIC ACTIVITY IN GLIOBLASTOMA

Devon McCourry[2,3], Sarah Stieglitz[2,4], Jonah Vilseck[2,4], Evan Cornett[1,2,3]

[1] *Center for Computational Biology and Bioinformatics, Indianapolis, IN*
[2] *Biochemistry and Molecular Biology Department*
[3] *Melvin and Bren Simon Comprehensive Cancer Center*
[4] *Center for Computational Biology and Bioinformatics*

Email: *dmccourr@iu.edu*

Glioblastoma multiforme (GBM) is the most common malignancy of the central nervous system in adults. GBM is classified as a WHO grade IV glioma and consists of the most aggressive, invasive, and undifferentiated type of glioma. This results in significant morbidity and mortality making the disease difficult to treat. Dysregulation of epigenetic regulators, including lysine methyltransferases (KMTs) and lysine demethylases (KDMs), contributes to a variety of cancer types, making them a promising target for cancer therapy. These enzymes modulate chromosome dynamics and protein-protein interactions through the methylation (KMTs) or demethylation (KDMs) of lysine residues on histone and non-histone substrates. SETD8 is a SET domain lysine methyltransferase known to monomethylate histone 4 on lysine 20 (H4K20me1). Alternatively, PHF8 is a JmjC domain-containing lysine demethylase shown to remove this mark[4]. Together, SETD8 and PHF8 play a vital role in regulating cell cycle progression by adding or removing H4K20me1, respectively. Not only does H4K20me1 regulate the cell cycle by altering gene expression but also serves as a mark for reader proteins of H4K20me1 to aid in transcriptional repression (L3MBTL1), DNA damage response (53BP1), and mitotic progression (Condensin II). SETD8 and PHF8 mRNA expression is positively correlated in cell lines and altering the balance between the two enzymes will shift levels of H4K20me1 and disrupt cell cycle progression. This work shows that inhibition of SETD8 with the substrate competitive inhibitor UNC0379 decreases cellular proliferation and causes cell cycle arrest in GBM cell lines, reinforcing the importance of SETD8 in the cell cycle. Although no chemical inhibitor of PHF8 has been developed to date, similar work has illustrated that upon RNA mediated knock down of PHF8, cells undergo defective mitosis and cell cycle arrest. Therefore, identifying the substrate selectivity profiles of SETD8 and PHF8 is a critical first step in deciphering their antagonistic functions on similar substrates and identifying their role in cell cycle and disease processes. To characterize the substrate selectivity of SETD8, we performed *in vitro* methyltransferase assays on recombinant substrates and peptides in solution. We show SETD8 is active on an unmodified H4K20 peptide and chicken mononucleosomes but fails to show activity on H4 protein alone. Simultaneously, our results show SETD8 prefers a C-terminal biotinylated peptide which will be used to validate a SETD8 substrate binding profile constructed via λ-dynamics with bias-updated Gibbs sampling (LaDyBUGS). Similarly, this work shows PHF8 is active on mononucleosomes via *in vitro* lysine demethylase assays. This preliminary work will help guide future studies of both enzymes' selectivity profiles and their antagonistic functions on H4K20me1 to better understand the role of SETD8 and PHF8 in disease processes.

Basic Science ***Graduate Student***

POSTER #25

COOPERATION OF YBX1 AND ETS TRANSCRIPTION FACTORS IN PROSTATE CANCER AND EWING SARCOMA

Samuel Metcalfe[1], Pete Hollenhorst[1]

[1] *Indiana University Bloomington, School of Medicine*

Email: *sametc@iu.edu*

Members of the ETS family of transcription factors (EF) are erroneously expressed in both prostate cancer and Ewing sarcoma after chromosomal rearrangements. In 65% of prostate cancers, one of four different EFs (ERG, ETV1, ETV4, ETV5), normally silent in prostate cells, become expressed after a fusion between the open reading frame of the EFs and the active promoters of genes expressed in prostate epithelia. These EFs form an essential complex with wild-type EWS to drive prostate cancer. In Ewing sarcoma, a chromosomal translocation leads to the expression of a fusion protein made of N-terminal EWS and an EF (usually FLI1 or ERG). This fusion protein and the EF-EWS complex in prostate cancer appear to have a common molecular mechanism. Our lab has identified a transcription factor YBX1 that interacts with EWS and some EFs. YBX1 has been implicated in multiple cancers and is upregulated in EF-driven prostate cancer, but its role in the EF-EWS complex in prostate cancer or with the EWS-fusion protein in Ewing sarcoma is unclear. I have performed preliminary experiments in prostate cancer cell lines that demonstrate that small-molecule inhibition of YBX1 leads to a decrease in EF-driven phenotypes. I aim to evaluate the role of YBX1 in ETS action in both prostate cancer and Ewing sarcoma. I will determine if the interactions between YBX1 and EWS and the EFs are direct or indirect, test how YBX1 knockdown and inhibition affects EF/EWS function, and determine if YBX1 has a role in EF-DNA binding. This research will improve our understanding of the molecular mechanism behind ETS-driven prostate cancer and Ewing sarcoma and may distinguish YBX1 as a target for cancer therapeutics.

Basic Science ***Graduate Student***

POSTER #26

CHARACTERIZING THE ROLE OF ZNF423 IN NEUROFIBROMATOSIS TYPE 1 (NF1)-RELATED MALIGNANT PERIPHERAL NERVE SHEATH TUMORS (MPNST).

Sarah Morrow[1,2,3], Shelley Dixon[1], Christopher Davis[1], Colin Beach[1,3], Christine Berryhill[1], Emily Zhang[1], Christine Pratilas[4], D. Wade Clapp[1], Steven Rhodes[1], Steven Angus[1]

[1] *Department of Pediatrics, IU School of Medicine, Indianapolis, IN*
[2] *Department of Pharmacology and Toxicology, IU School of Medicine, Indianapolis, IN*
[3] *Simon Comprehensive Cancer Center, IU School of Medicine, Indianapolis, IN*
[4] *Johns Hopkins University, School of Medicine, Baltimore, MD*

Email: *morrowsa@iu.edu*

Purpose: Recent work in our lab utilized transcriptomics to analyze two *SUZ12*-deficient human MPNST cell lines with restored PRC2 activity, which revealed downregulation of *ZNF423*, a lineage-specific transcription factor. These results were validated in murine tumor cells-of-origin isolated from GEMMs of plexiform neurofibroma (PNF) and MPNST, which revealed *ZNF423* was significantly upregulated in MPNST compared to both wild-type controls and PNF, suggesting a potential role for this protein in MPNST tumorigenesis. Our goal is to identify potential therapeutic targets for NF1-related MPNST by elucidating ZNF423-dependent signaling mechanisms that may influence MPNST lineage, identity and proliferation.

Methods: Human MPNST cell line models were employed for RNA interference, RNA sequencing, immunoblotting, proteomic analysis of the kinome, cell-based assays, and *in vivo* implantation.

Results: Short-interfering RNA (siRNA) depletion of *ZNF423* from four human MPNST cell lines altered MPNST cell morphology, and significantly decreased cell proliferation and viability compared to controls. EdU/DAPI staining further confirmed these effects, showing a marked decrease in DNA synthesis and hence impaired cell cycle progression and proliferation in ZNF423 knockdown cells. To establish the biological mechanism underlying these phenotypic effects, we performed RNA sequencing, which revealed significant transcriptional changes, with overlapping gene expression patterns amongst the four cell lines. Shared significantly downregulated genes were enriched for pathways involved in proliferation, migration and development. In contrast, shared upregulated genes were enriched for pathways involved in UV response, DNA damage checkpoint activation and cell cycle arrest, suggesting a shift toward stress-induced inhibition of tumor growth. To assess the effects of ZNF423 knockdown *in vivo*, we generated a human MPNST cell line with stable *ZNF423* suppression using shRNA, which was implanted into the sciatic nerve of NRG mice. Tumor growth was measured over time revealing that ZNF423 knockdown led to marked reduction in tumor size compared to controls, and individual tumor growth curves show a consistent slower progression in the ZNF423 knockdown mice.

Conclusion: These studies suggest that ZNF423 regulates key pathways that maintain MPNST survival, as depletion reduces MPNST cell viability and interferes with tumor growth *in vivo*. Ongoing studies will further delineate ZNF423-dependent signaling pathways using ChIP-seq, affinity purification-mass spectrometry (AP-MS), and multiplex inhibitor bead mass spectrometry (MIB/MS) kinome profiling to reveal druggable targets within these pathways.

Basic Science ***Graduate Student***

POSTER #27

NEUROMUSCULAR DYSFUNCTION PRECEDES MUSCLE ATROPHY AND BONE LOSS IN A MURINE MODEL OF MALE COLORECTAL CANCER

Paola Ortiz Gonzalez[1,5], Fabiola Galiana-Melendez[2], Davis Giffin[2], Morgan Clouse[2], Fabrizio Pin[3], Lilian Plotkin[4], Joshua Huot[3]

[1] *Indiana Center for Musculoskeletal Health, Indianapolis, IN*
[2] *Indiana University School of Medicine, Indianapolis, IN*
[3] *Indiana University School of Medicine, Indiana Center for Musculoskeletal Health, Simon Comprehensive Cancer Center, Indiana University School of Medicine, Department of Anatomy, Cell Biology and Physiology, Indianapolis, IN*
[4] *Indiana University School of Medicine, Indiana Center for Musculoskeletal Health, Department of Anatomy, Cell Biology and Physiology, Richard L. Roudebush Veterans Administration Medical Center, Indianapolis, IN, USA, Indianapolis, IN*
[5] *Indiana University School of Medicine*

Email: *portizgo@iu.edu*

Cancer cachexia, characterized by skeletal muscle wasting and weakness, worsens quality of life and accounts for up to 30% of cancer-related deaths. Although detection and treatment advancements have increased cancer survivorship, muscle dysfunction can persist long after remission. Cachexia also accelerates bone resorption and reduces bone formation, leading to bone loss and compromised strength. We previously demonstrated that late-stage cachexia impairs skeletal muscle innervation, linking motor unit loss to muscle wasting and weakness. Here, we examined the early onset of neuromuscular dysfunction and bone loss in a preclinical colorectal cancer model. 8-week-old CD2F1 male mice were injected subcutaneously with murine C26 cancer cells or saline and assessed at 6, 8, or 10 days (n=8-10/group) for indices of motor unit connectivity, muscle contractility, bone geometry and strength. Skeletal muscles were harvested, weighed, and processed for molecular analyses; tibiae were collected and used for μCT. At 6 days post-injection, C26 hosts exhibited reductions in neuromuscular junction (NMJ) transmission and motor unit connectivity, while muscle mass and absolute muscle torque remained intact. Specific muscle torque declined by 8 days, with significant muscle mass and cross-sectional area loss emerging at 10 days. Molecular analysis revealed NMJ alterations at 6 days, indicating that neuromuscular dysfunction precedes muscle atrophy. In bone, elevated levels of C-terminal telopeptide (CTX) at 8 and 10 days indicate increased bone resorption, preceding muscle atrophy. μCT analysis of tibiae showed reduced trabecular BV/TV and Tb.N and increased Tb.Sp at 10 days, although cortical bone mass and femoral biomechanical properties remained unchanged. Our findings suggest that cancer-induced NMJ dysfunction is an early predictor of cachexia progression and muscle atrophy. Deterioration of trabecular bone architecture may result from impaired mechanical loading due to reduced muscle function. Strategies targeting neuromuscular preservation could mitigate cachexia progression and enhance quality of life for cancer patients and survivors.

Basic Science ***Graduate Student***

POSTER #28

PP2A-B56A ACTIVATION DRIVES EGFR SIGNALING IN PANCREATIC CANCER

Claire Pfeffer[1,2], Sydney Clifford[1,2], Ella Rose Chianis[1,2], Brittany Heil[1,2], Emma Kay[1,2], Garima Baral[1,2], Elizabeth Hoffman[1,2], Saadia Karim[3], Jukka Westermarck[4], Jennifer Morton[5,6], Brittany Allen-Petersen[1,2]

[1] *Department of Biological Sciences, Purdue University*
[2] *Purdue Institute for Cancer Research, Purdue University*
[3] *CRUK Beatson Institute*
[4] *Turku Bioscience Centre, University of Turku*
[5] *UK Beatson Institute*
[6] *School of Cancer Sciences, University of Glasgow*

Email: *cpfeffe@purdue.edu*

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 #29

TARGETING THE BAF CHROMATIN REMODELING COMPLEX IN CASTRATION-RESISTANT PROSTATE CANCER

Jude Tetteh Quarshie[1,2], Masataka Nakano [2], Emily Dykhuizen[1,2]

[1] Borch Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907, USA.
[2] Purdue Institute for Cancer Research, Purdue University, West Lafayette, IN 47906, USA.

Email: *jquarshi@purdue.edu*

Prostate cancer (PCa) remains the most prevalent cancer type among men worldwide. Although existing PCa treatment strategies have been beneficial, drug resistance significantly limits their success. Evidence shows that androgen receptor (AR)-mediated transcription drives drug-resistant PCa and depends on co-regulatory proteins like BAF (SWI/SNF) complexes. BAF complexes are multidomain chromatin remodelers that have been found to recruit AR, increase chromatin accessibility for AR binding, and/or enhance AR transactivation. Surprisingly, althoughBAF complexes are altered in over 20% of cancers, BAF mutations are rarely found in PCa, which may imply a requirement for intact BAF in PCa. As such, some studies have assessed the sensitivity of various in vitro and in vivo PCa models to inhibitors or degraders of BAF subcomplexes, as a potential treatment modality for PCa. We previously described a 12-membered macrolactam scaffold (named BD98) which interacts with the canonical BAF (cBAF) sub-complex. Data from our group demonstrates that BD98 reduces the expression of mutant AR (i.e.AR-V7) and its target genes without affecting wild-type AR expression. Furthermore, BD98 targets signaling pathways similar to enzalutamide; suggesting that BD98 may be a novel chromatin-targeted therapeutic against AR-V7-driven PCa.

Basic Science ***Graduate Student***

POSTER #30

TRYPTOPHAN METABOLISM PLAYS A SIGNIFICANT ROLE IN MULTIPLE MYELOMA SURVIVAL

Julia Grace Reinke[5], Kanita Chaudhry[1], Peng Peng[1], Louise Carlson[2], Daniela Petrusca[2], Chris Schorr[3], Kelvin Lee[4]

[1] *Roswell Park Comprehensive Cancer Center Department of Immunology, , IN*
[2] *Indiana University School of Medicine Division of Hematology and Oncology, , IN*
[3] *Purdue University Department of Biomedical Engineering , , IN*
[4] *Indiana University Simon Comprehensive Cancer Center, , IN*
[5] *Indiana University School of Medicine, Department of Microbiology and Immunology*

Email: *jgreinke@iu.edu*

Multiple myeloma (MM) is a hematological malignancy caused by abnormally proliferating plasma cells in the bone marrow. It is considered incurable, as patients almost always go through cycles of treatment, remission and treatment-resistant relapse. Early stage MM depends on the Bone marrow Microenvironment (BMME) for survival. We have previously shown that MM interacts with BMME Dendritic Cells (DC) which induces DC production of Indolamine Dioxygenase 1 (IDO1), which catabolizes tryptophan (TRP) to kynurenine (KYN). Depletion of TRP suppresses T effector cell activation and the production of KYN activates pro-survival pathways and MM through the activation of the transcription factor Aryl Hydrocarbon Receptor (AHR). We now show that MM cells can produce KYN independent of DCs through expression of the TRP catabolizing enzyme Tryptophan Dioxygenase (TDO), indicating a mechanism by which MM can become independent of the BMME. High TDO expression negatively impacts patient survival outcomes. Inhibiting TDO with non-competitive inhibitor 680C91 or knocking down TDO significantly reduced MM cell survival in TDO+ MM cell lines. Inhibiting TDO reduced KYN production and AHR activation in TDO+ MM cell lines, but did not affect a TDO- MM cell line. Co-culture with IDO1+ DCs or treatment with AHR ligand TCDD rescued MM cell viability from TDO inhibitor-induced cell death, indicating that TRP metabolism to KYN is important to MM survival. MM depends on different methods of TRP metabolism for survival both in the BMME and as it becomes independent of it. TRP metabolism is a novel treatment target in MM and could lead to more effective cell killing and immunotherapy, especially in relapse/refractory disease.

Basic Science ***Graduate Student***

POSTER #31

TARGETING REPLICATION DYNAMICS: PARP1 AS A REGULATOR OF DNA POLYMERASE FUNCTION IN THE CONTEXT OF PARPI

Daniela Samuel[3], Pam VanderVere-Carroza[1], John Turchi[1], Lata Balakrishnan[2]

[1] Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202 , Indianapolis, IN
[2] Department of Biology, Indiana University Indianapolis, Indianapolis, IN 46202, Indianapolis, IN
[3] Department of Biology, Indiana University Indianapolis, Indianapolis, IN 46202

Email: *dfsamuel@iu.edu*

PARP inhibitors (PARPi) are an established therapeutic used to treat severe cases of BRCA1/BRCA2-mutated cancers. While these inhibitors provide a targeted and effective option when used in conjunction with other chemotherapeutic agents, many patients develop resistance after prolonged exposure, compromising long-term efficacy. Key early cellular responses include an increased replication fork speed and an accumulation of single-stranded DNA (ssDNA) gaps that arise from dysregulation of lagging strand maturation upon PARPi treatment. One key determinant of PARPi sensitivity is the relationship between PARP1 and DNA polymerases, as demonstrated by previousin vivo studies. Despite extensive cell-based assays, how PARP1 interacts with DNA replication and repair polymerases in a defined, in vitro system remains unclear. PARP1 influences protein interactions and activity through mechanisms such as DNA binding and covalent modification of target proteins. Auto-modification of PARP1 is known to decrease its affinity for DNA. Our first hypothesis is that unmodified and PARPi-treated PARP1 may inhibit polymerase activity through persistent DNA association. We further propose that direct PARP1-mediated modification of DNA polymerases δ, ε, and β may alter their activity. Together, we propose that PARP1 will regulate the catalytic activity and processivity of DNA polymerases δ, ε, and β in vitro. By examining PARP1's interactions with DNA polymerases under both native and PARPi-treated conditions, we aim to further clarify early cellular responses to PARPi. These insights may identify new therapeutic targets to restoresensitivity to resistant cancer cells.

Basic Science ***Graduate Student***

POSTER #32

SYNERGISTIC EFFECT OF PARP INHIBITORS AND ENZALUTAMIDE ON AR ACTIVITY IN PROSTATE CANCER

Mallory Sands[1,4], Michael Li[2], Leo Leon[2], Adriana Zablah[2], Jianneng Li[3]

[1] *Harper Cancer Research Institute, University of Notre Dame, Notre Dame, IN*
[2] *Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, Harper Cancer Research Institute, University of Notre Dame, Notre Dame, IN*
[3] *Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, Harper Cancer Research Institute, University of Notre Dame, Notre Dame, IN, Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, Indiana*
[4] *Department of Biological Sciences, University of Notre Dame, Notre Dame, IN*

Email: *msands2@nd.edu*

Prostate cancer is the most common malignancy and the second leading cause of cancer-related deaths in men in the United States. Its progression is driven by androgen-activated androgen receptor (AR), making almost all cases initially sensitive to androgen deprivation therapy. However, despite the initial effectiveness, the cancer often recurs as castration-resistant prostate cancer (CRPC), a highly lethal form that remains AR-dependent. This has led to the development of second-generation antiandrogens, such as enzalutamide, which can further inhibit AR activation. Unfortunately, while patients initially respond to enzalutamide, nearly all develop treatment-resistant disease through various mechanisms, including the emergence of AR mutations, AR variants, and homologous recombination repair mutations (HRRm). At this advanced stage, poly (ADP-ribose) polymerase inhibitors (PARPis) function by trapping PARP at single-stranded DNA breaks, leading to replication fork collapse and double-stranded DNA breaks. In cells deficient in homologous recombination repair, this results in cell death. Consequently, PARPis are most effective in tumors with HRRm, and have recently been approved for patients carrying these mutations. Furthermore, A recent clinical trial evaluated the combination of enzalutamide and the PARPi talazoparib as a first-line treatment in CRPC, and found improved outcomes, even in patients without HRRm. However, the mechanism underlying this synergy remains unclear. In HRRm-free prostate cancer cell lines, our data shows that both enzalutamide and PARPis individually suppress AR signaling in hormone-containing culture medium. However, only PARPis suppress AR signaling in hormone-free conditions, while only enzalutamide inhibits androgen-induced AR signaling. Moreover, their combination further suppresses AR signaling beyond either drug alone. Notable, PARPis also suppresses AR variants and mutations, which enzalutamide does not affect. Collectively, these findings suggest that enzalutamide and PARPis inhibit AR signaling through complementary mechanisms, contributing to their synergistic effect and supporting the clinical use of this combination therapy.

Basic Science ***Graduate Student***

POSTER #33

THE ROLE OF ZEB1 IN LINEAGE SPECIFICITY AND CELL FATE CONVERSION OF AFRICAN-ANCESTRY ENRICHED BREAST MULTIPOTENT STROMAL CELLS

Madhura Shirish Shukla[4], Maya Krishnan[1], Michelle Niese[1], Jia Ji[2], Hongyu Gao[2], Yunlong Liu[2], Mark Kaplan[1], Harikrishna Nakshatri[3]

[1] *Department of Microbiology & Immunology, IU School of Medicine, Indianapolis, IN*
[2] *Department of Medical and Molecular Genetics, IU School of Medicine, Indianapolis, IN*
[3] *Department of Surgery, IU School of Medicine, Richard L Roudebush VA Medical Center, Indianapolis, IN*
[4] *Indiana University Simon Comprehensive Cancer Center, Indiana University School of Medicine, Indianapolis*

Email: *madshukl@iu.edu*

Disparity in breast cancer outcomes in women of African Ancestry (AA) as compared to European Ancestry (EA) has been thought to derive from socioeconomic factors. However, emerging evidence demonstrating genetic ancestry-dependent differences in normal and cancer genome, cancer-associated mutation patterns and immunosuppressed tumor microenvironment suggests the involvement of biological factors. To investigate breast cancer outcome-associated biologic factors, we previously analyzed breast tissues of healthy donors of distinct genetic ancestry. We found that PROCR+/ ZEB1+/ PDGFRα+ stromal cells (hence, called PZP) are more abundant in normal breast tissues of women of AA as compared to EA. PZP cells display the properties of multi-lineage fibroadipogenic mesenchymal stromal cells that can differentiate into adipogenic and osteogenic lineages. Mechanistic studies of PZP cells in the context of breast cancer initiation and progression are yet to be reported. This study focused on the PZP cell enriched Zinc finger E-box-binding homeobox 1 (ZEB1), which is a master transcription regulator of stemness and epithelial-to-mesenchymal transition (EMT). We generated ZEB1 gene knockout clones of PZP cells using CRISPR-Cas9 and performed RNA-sequencing of ZEB1 knockout clones and parental cells. The expression of epithelial markers KRT 7, 8, 18 and adipogenic markers PPARG and PPARGC1B were upregulated upon ZEB1 knockout. Additionally, ZEB1-deficiency increased the levels of inflammatory fibroblast markers (iCAFs), while downregulating the expression of myofibroblast marker genes encoding extracellular matrix proteins Tenascin-C (TNC), matrix metallopeptidase 1 (MMP1) and a surface protein, Leucine-rich repeat-containing protein 15 (LRRC15). These results suggest the role of ZEB1 in lineage specificity and cell fate conversion of PZP cells and shape the normal and breast tumor microenvironment. Consistent with this possibility, ZEB1 controlled the expression of prostaglandin-endoperoxide synthase 1 (PTGS1), which synthesizes immunosuppressive prostaglandin E2 (PGE2), in PZP cells. In support of the immunosuppressive role of PZP cells, PZP cell secreted factors reduced the fraction of Interferon γ and Granzyme B expressing CD8+ T cells in an in vitro T cell activation assay. Ongoing studies are investigating the potential contributions of ZEB1, PGE2, and TNC in the PZP cell-mediated decrease of CD8+ T cell effector functions. Thus, ZEB1 is a multifunctional transcription factor that controls various biochemical networks in the breast tumor microenvironment through PZP cell-intrinsic and extrinsic activities and is a candidate for therapeutic intervention.

Basic Science ***Graduate Student***

POSTER #34

LOSS OF GCN2 EIF2 KINASE SENSITIZES PROSTATE CANCER CELLS TO DEPLETION OF POLYAMINES

Noah Sommers[3], Ricardo Cordova[1], Ronald Wek[2], Kirk Staschke[2]

[1] *Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Princeton University, Indianapolis, IN*

[2] *Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN*

[3] *Department of Biochemistry and Molecular Biology, Indiana University School of Medicine*

Email: *noahsomm@iu.edu*

The Integrated Stress Response (ISR) features multiple protein kinases that each sense distinct cellular stresses, phosphorylating eIF2 to direct gene expression that serves to mitigate cell damage and restore homeostasis. Previously, we reported that the eIF2 kinase GCN2 and the ISR are constitutively active in prostate cancer (PCa) and required to maintain amino acid (AA) homeostasis to sustain tumor growth (Cordova et al., 2022, *eLife*). GCN2 is induced by nutrient limitation, and basal GCN2 activation in PCa enhances the expression of transporters that ensure sufficient AAs are available for PCa proliferation. However, while genetic or pharmacological inhibition of GCN2 sharply reduces AAs and growth of PCa cells in culture and mouse models, there is minimal cell death.

We hypothesized that signaling pathways function to support cell survival in GCN2-deficient PCa cells and can be targeted to promote cell death in combination therapies. Indeed, we discovered that loss of GCN2 leads to compensatory activation of p53 signaling that blocks cell growth and lowers nutrient expenditure (Cordova & Sommers et al., 2024, *Science Signaling*). In this recent report, we also carried out a targeted CRISPR-interference screen in castration-resistant 22Rv1 PCa cells with functional wild type (WT) GCN2 or in cells deleted for GCN2 (GCN2 KO). We discovered that reduced expression of ornithine decarboxylase (ODC), the rate-limiting enzyme for polyamine (PA) biosynthesis, was synthetically lethal with loss of GCN2. PAs are metabolites derived from AAs that are ubiquitous among tissues, but especially abundant in prostate tissue. Given that PA biosynthesis requires AAs and GCN2 maintains AA homeostasis in PCa, we propose that GCN2 regulates PA levels in PCa. Indeed, intracellular levels of both AAs and PAs were decreased in GCN2-inhibited LNCaP cells compared to cells treated with vehicle alone.

We sought to determine whether defects in PA biosynthesis and metabolism also contribute to the growth defect of GCN2-deficient cells. To investigate whether GCN2-deficient cells are sensitive to PA depletion, we treated WT or GCN2 KO 22Rv1 organoids with DFMO, a small molecule inhibitor of ODC. While inhibition of ODC resulted in minimal death of WT cells, there was induced death in GCN2 KO cells. One of the best characterized functions of PAs involves PA-derived hypusination of the translation factor eIF5A, a post-translational modification of eIF5A that is required for efficient translation of mRNAs encoding polyproline tracts. Interestingly, we found that GCN2 promoted eIF5A hypusination and enhanced the expression of several genes associated with PA metabolism in 22Rv1 cells. Of note, eIF5A hypusination inhibition resulted in the selective death of GCN2-deficient cells in a 22Rv1 organoid model. Our study suggests that GCN2 maintains PAs in PCa cells and combined therapies targeting GCN2 and PA biosynthesis are an effective strategy for PCa treatment.

Basic Science ***Graduate Student***

POSTER #35

CHARACTERIZATION OF A NOVEL SMALL MOLECULE INHIBITOR OF ASPARAGINE SYNTHETASE

Nicholas Walda[1,2], Wen Zhu[3], Nigel Richards[4], Yuichiro Takagi[2], Ronald Wek[1,2], Kirk Staschke[1,2]

[1] *Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN*
[2] *Department of Biochemistry and Molecular Biology, Indiana University School of Medicine*
[3] *Department of Chemistry and Biochemistry, Florida State University*
[4] *Foundation for Applied Molecular Evolution (FfAME)*

Email: *nwalda@iu.edu*

Nutrients can become limiting in many cancers due to restrictive tumor microenvironments. In response to these limitations, GCN2 protein kinase and the Integrated stress response (ISR) induce genes involved in the uptake and synthesis of amino acids that assist in ensuring the maintenance of amino acids and the progression of tumors. Activation of GCN2 by amino acid limitation induces phosphorylation of the eukaryotic initiation factor 2 (eIF2) to regulate translation initiation. Phosphorylation of eIF2 reduces global translation but leads to preferential translation of select mRNA transcripts, such as those encoding the bZIP transcription factor ATF4. The *ASNS* gene is a direct target gene of ATF4, and GCN2 is critical for the expression of ASNS in many cancers, making the GCN2-ATF4-ASNS signaling pathway a promising target for cancer therapy.

Historically, ASNS has been a challenging therapeutic target, as most inhibitors developed to date lack efficacy in cell-based systems. Recently, a series of substituted pyrrolidines were identified as potent ASNS inhibitors. Among these, compound 17 (also known as ASX-173) was characterized using a combination of biochemical and cellular assays. Using recombinant ASNS expressed and purified from insect cells, ASX-173 inhibited asparagine synthesis with an IC_{50} of 240 nM. Differential scanning fluorimetry (DSF) experiments revealed that ASX-173 binding to ASNS depends on the presence of Mg^{2+}-ATP. Consistent with this finding, the cryo-EM structure of the ASNS:ASX-173 complex determined at 3.5 Å resolution showed that AMP bridges ASNS and ASX-173, and that ASX-173 occupies the synthetase active site, aligning with the position normally occupied by the β-aspartyl-AMP intermediate derived from ATP and aspartate. These findings suggest that ASX-173 may function as an uncompetitive inhibitor of ASNS.

Treatment of HEK293A cells cultured in the absence of asparagine potently induced the ISR, resulting in activation of GCN2, increased phosphorylation of eIF2, increased expression of ATF4, and increased ATF4 transcriptional activity. Importantly, the effects of ASX-173 on the ISR were readily reversed by adding L-asparagine to the culture media, indicating the specificity of ASX-173 for ASNS. ASX-173 inhibited the growth of 293A (kidney), RENCA (kidney), and B6Myc-CaP (prostate) cancer cell lines when grown in the absence of asparagine. Significantly, treatment of these cells with ASX-173 in combination with GCN2iB, a small molecule inhibitor of GCN2, resulted in synergistic growth inhibition. Together, our results suggest that targeting the GCN2-ATF4-ASNS signaling axis warrants further study for developing novel combination treatments for cancer, including prostate and kidney cancer.

Basic Science ***Graduate Student***

POSTER #36

THE ROLE OF SGK1 IN GLUCOSE METABOLISM TO EVADE CELL DEATH IN ECM-DETACHED CELLS

Lauren Young[1]

[1] *University of Notre Dame*

Email: *lyoung5@nd.edu*

The ability of cancer cells to detach from the extracellular matrix (ECM) and inhabit distant regions of the body accounts for 90% of cancer mortalities. While detachment from the ECM

induces caspase-dependent cell death, metastatic cancer cells undergo metabolic changes to circumvent death pathways such as anoikis. Cancer cells can acquire resistance to anoikis by activating pro-survival signaling pathways such as the PI(3)K signaling cascade. Many studies on effectors of PI(3)K signaling in cell proliferation heavily focus on AKT. Previous work in our lab discovered that serum and glucocorticoid kinase 1 (SGK1), a kinase with significant homology to AKT, is a driver of metabolic changes that promote survival during ECM-detachment. While AKT has been well-studied during tumorigenesis, it's becoming clear that SGK1 may also have a significant role in tumor progression and metastasis. This work seeks to understand how changes in the environmental composition of metabolites in the environment using physiologic media and glucose starved conditions will alter the capacity of SGK1 to mediate glucose metabolism. Using multiple cancer cell lines engineered to express constitutively active SGK1, our results demonstrate that SGK1 is more effective than AKT in promoting GLUT1 trafficking and activity in ECM-detached cells. In summary, these findings establish SGK1 as a key regulator of glucose uptake, offering new insights into how cancer cells adapt and survive beyond their native microenvironment.

Basic Science ***Graduate Student***

POSTER #39

GENOMIC DETERMINANTS OF PIK3CA MUTATION-DRIVEN BREAST CANCER INITIATION

Snehal Bhandare[2,3,4], Reggie Wang[4,2], Poornima Nakhastri[4,2], Stephanie Adama[1,3], Harikrishna Nakshatri[2,3,4]

[1] *Indiana University School of Medicine, Indianapolis, IN*
[2] *Department of Surgical Oncology*
[3] *Simon Comprehensive Cancer Center*
[4] *Indiana University School of Medicine*

Email: *snebhan@iu.edu*

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. Mutations in PIK3CA are found in many normal organs including the breast suggesting that additional genomic aberrations are needed for PIK3CA to initiate breast cancer, and those aberrations are therapeutic targets. Consistent with this possibility, we had previously shown that mutant PIK3CA can transform breast epithelial cells from healthy women only when combined with SV40-T/t antigens. However, breast epithelial cells from germline BRCA1/2 mutation carriers are susceptible for mutant PIK3CA mutation driven transformation. From these results, we hypothesized that genomic aberrations that render BRCA1/2 dysfunctional can substitute for SV40-T/t antigens and cooperate with mutant PIK3CA to transform breast epithelial cells. In fact, several groups have been searching SV40-T/t antigen mimicking genomic aberrations for more than three decades. To test our hypothesis, we first compared signaling networks in mutant PIK3CA±SV40-T/t antigens overexpressing breast epithelial cells from healthy donors, BRCA1 and BRCA2 mutation carriers and observed elevated activity of MEK/ERK and NF-kB either at basal level or upon mutant PIK3CA overexpression in BRCA1 and BRCA2 mutant cells but activation of these pathways in breast epithelial cells of healthy donors required overexpression of both PIK3CA and SV40-T/t antigens. Examination of breast cancer genomic data showed a significant number of breast cancers with PIK3CA mutations accompanied by loss-of-function of NF-1 mutations or reduced expression of DAB2IP, both of which can lead to MEK/ERK activation, and amplification of IkBKB or IkBKE, which can lead to NF-kB activation. To determine which among these pathways are needed for mutant PIK3CA-driven breast cancer initiation, we overexpressed IkBKB, IkBKE or constitutively active MEK1 along with mutant PIK3CA in breast epithelial cells and examined these cells for tumorigenicity in NSG mice. While mutant PIK3CA plus IkBKB or IkBKE overexpressing cells failed to generate any tumors, cells overexpressing mutant PIK3CA plus MEK1 generated tumors. Constitutively active MEK1 alone displayed mild oncogenic activity. We also discovered a role for IkBKB in metastatic progression of mutant PIK3CA driven breast cancer. These results suggest that mutant PIK3CA is a weaker oncogene and effective therapy for mutant PIK3CA driven breast cancers needs to include inhibitors of PIK3CA as well as drugs that target genomic aberrations that cooperate with mutant PIK3CA.

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

POSTER #40

EFFECT OF 17β-ESTRADIOL ON CELL PROLIFERATION AND PD-L1 GENE EXPRESSION IN NON-SMALL CELL LUNG CANCER CELL LINES

Omar Borges-Sosa[2], Patricia Silveyra[1]

[1] *Department of Environmental and Occupational Health, School of Public Health Bloomington, Bloomington, IN*
[2] *Department of Environmental and Occupational Health, School of Public Health Bloomington*

Email: *oborges@iu.edu*

Background: Lung cancer is the leading cause of cancer-related deaths worldwide. Emerging evidence suggests that 17β-Estradiol (E2) may influence tumor proliferation, while estrogen receptors (ER) may predict therapy response and prognosis in non-small cell lung cancer (NSCLC). However, the specific roles of each type of ER in NSCLC are still unclear. In this study, we aimed to evaluate the proliferation and gene expression of two NSCLC cell lines that are known to exhibit different levels of mRNA ESR1 gene expression and ER alpha protein expression. We assessed these variables after exposing them to either E2 or vehicle control.

Methods: *MTT Assay:* We seeded LL/2-Luc2 and A549 cells in an estrogen-free medium in 96-well plates. E2 was diluted in the medium to achieve seven concentrations, while a vehicle was used as a control. The MTT assay was conducted after the exposure, following the established protocol to obtain optical density values as a measure of cell proliferation. The results were normalized to the vehicle control. *RNA Isolation and qPCR:* We seeded A549 cells in an estrogen-free medium in 6-well plates. E2 or a vehicle was diluted in the medium. RNA was isolated using TRIzol reagent and then reverse transcribed to cDNA using the High-Capacity cDNA Reverse Transcription Kit, following established protocols. TaqMan assays were used to determine the expression levels of PD-L1, EGFR, and VEGF genes. Relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method normalized to 18S.

Results: Our results showed a biphasic effect on cell proliferation following exposure to E2. In LL/2-Luc2 cells, we observed a significant increase in proliferation after E2 treatment. Conversely, in A549 cells, a significant decrease in proliferation was noted. Additionally, E2 exposure in A549 cells significantly reduced PD-L1 gene expression compared to the vehicle-exposed cells. No significant differences were found in EGFR and VEGF gene expression in A549 cells.

Conclusion: These preliminary data suggest that E2 may enhance cell proliferation according to the status of the ER alpha. Furthermore, E2 may play a role in regulating PD-L1 gene expression in NSCLC. Ongoing gene expression experiments with LL/2-Luc2 cells will provide further insights. Future work will focus on validating these results.

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

POSTER #41

REGULATORY LONG NONCODING RNA OF T CELLS (RELOT/LINC00402) DECREASES ALLOGENEIC T CELL-DRIVEN ACUTE GRAFT-VERSUS-HOST DISEASE

Van Anh Do-thi[5], Emm Bratch[1], Jathniel Hernandez[1], Dani Jade-Alice Bastaic[2], George Sandusky[3], Daniel Peltier[4]

[1] Division of Pediatric Hematology, Oncology, and Stem Cell Transplantation, Department of Pediatrics, Herman B. Wells Center for Pediatric Research, Simon Cancer Center, Indiana University School of Medicine, Indianapolis, IN
[2] Department of Pathology and Laboratory Medicine, Simon Cancer Center, Indiana University School of Medicine, Indianapolis, IN
[3] Department of Pathology and Laboratory Medicine, Simon Cancer Center, Indianapolis, IN
[4] 1Division of Pediatric Hematology, Oncology, and Stem Cell Transplantation, Department of Pediatrics, Herman B. Wells Center for Pediatric Research, Simon Cancer Center, Indiana University School of Medicine, Indianapolis, IN
[5] Division of Pediatric Hematology, Oncology, and Stem Cell Transplantation, Department of Pediatrics, Herman B. Wells Center for Pediatric Research, Simon Cancer Center, Indiana University School of Medicine

Email: *vatdo@iu.edu*

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment for high-risk hematologic malignancies. 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/LINC00402*) as a biomarker for aGVHD. However, *LINC00402's* function in allogeneic T-cell driven aGVHD remains unknown.

To determine *LINC00402's in vivo* function, we established a *Rosa26* knock-in T cell conditional overexpression C57BL/6 (B6) mouse model. To determine whether *LINC00402* decreases allo-T cell-driven aGVHD, B6 *LINC00402* overexpressing or control T cells were transferred into lethally irradiated major histocompatibility complex (MHC) mismatched BALB/c recipients along with wild-type (WT) B6 T cell-depleted bone marrow cells. Recipients of *LINC00402* overexpressing donor T cells had significantly improved survival relative to recipients of control donor T cells (p=0.007). This phenotype was model-independent because *LINC00402* overexpressing donor allo-T cells improved overall survival (p=0.003), aGVHD clinical score (p=0.005), and weight loss (p=0.03) in MHC-matched but minor histocompatibility antigen-mismatched C3H.sw recipient mice.

Acute GVHD-related mortality is most closely associated with gastrointestinal (GI) and hepatic GVHD. Histopathological analysis of the GI tract and liver in BALB/c recipients indicated that mortality in our model was largely due to GI tract damage, with the protective effect of *LINC00402* most prominent in the small intestines (p=0.0002).

To elucidate the mechanism underlying *LINC00402's* protective effect, we analyzed the accumulation of donor T cell subsets known to either promote or inhibit aGVHD. In the spleen, there was no difference in bulk CD3+ donor T cells nor in donor naïve, central memory, effector memory, Th1/Tc1, Th2/Tc2, or Treg cells. By contrast, accumulation of aGVHD-promoting donor Th17/Tc17-like cells (i.e., CD4+ Rorgt+ and CD8+ Rorgt+ cells) decreased in *LINC00402* overexpressing allo-T cell recipients (p=0.004 CD4+ and p=0.004 CD8+). This effect was likely direct because *in vitro* Th17 polarization was decreased by *LINC00402* (p=0.007). In contrast to the spleen, bulk CD3+ donor T cell accumulation was reduced in the small intestinal epithelium (p=0.04). These results suggested that in addition to Th17 polarization being impaired, trafficking of Th17-like cells to the GI tract may also be impaired. Consistent with this, expression of the chemokine receptors Cxcr3 and Ccr6, both of which are associated with Th17/Tc17 cells and known to aggravate allo-T cell-driven GI aGVHD, were reduced in spleen and intestinal epithelial *LINC00402* overexpressing Rorγt+ donor T cells. Analysis of serum Cxcr3 and Ccr6 ligands showed significantly reduced concentration of the chemokine Cxcl9 (p=0.03), a Cxcr3 ligand, likely further contributing to impaired trafficking of *LINC00402* overexpressing donor T cells.

Granzyme B and perforin are critical components of allo-T cell cytotoxicity and damage of host intestinal tissue. *LINC00402* overexpression decreased expression of granzyme B in small intestinal intraepithelial Rorγt+ CD4+ T cells (p=0.002), whereas perforin was unaffected. Altogether, our results demonstrated that *LINC00402* restrains allo-T cell-driven aGVHD by impairing Th17 polarization, GI tract homing, and cytotoxic function. Developing strategies to preserve *LINC00402* expression or enhance its down-stream functions may improve aGVHD-related outcomes.

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

POSTER #42

SPATIAL TRANSCRIPTOMICS UNVEILS TUMOR MICROENVIRONMENT CHANGES IN MURINE GLIOBLASTOMA UNDER TREATMENT

David Eisenbarth[1], Jun Wan[2], Sheng Liu[2], Juexin Wang[3], Shuang Wang[3], Y. Alan Wang[1]

[1] the Brown Center for Immunotherapy, Department of Medicine, Melvin and Bren Simon Comprehensive Cancer Center, Indiana University School of Medicine, Indianapolis, IN 46202, USA
[2] Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN 46202, USA
[3] Department of BioHealth Informatics, Luddy School of Informatics, Computing, and Engineering, Indiana University Indianapolis, Indianapolis, IN 46202, USA

Email: *deisenb@iu.edu*

Glioblastoma (GBM) is one of the deadliest types of cancer, with a 5-year survival rate well below 10%, and treatment options are severely limited. Two defining characteristics of GBM are its heterogeneity and immunosuppressive tumor microenvironment (TME), which is largely driven by anti-inflammatory macrophages. Together, these factors result in a negligible response rate to immune checkpoint blockade (ICB), leaving patients with few alternatives. Recent studies have shown that inhibition of TANK-binding kinase 1 (TBK1) increases the response rate to PD-1 blockade in melanoma. In GBM, TBK1 inhibition leads to the repolarization of tumor-associated macrophages (TAMs) from an immunosuppressive to an inflammatory phenotype. Advances in imaging and single-cell technologies now allow for the quantification and visualization of gene expression while preserving the spatial organization of tissues. The current generation of spatial transcriptomics (ST) can achieve single-cell resolution, opening up new possibilities for understanding the intricate network of tumor and immune cell interactions and how these change following therapeutic intervention. To investigate the effect of TBK1 inhibition in a mouse model of GBM, we performed ST using 10X Visium HD. Following quality control, we identified 21 clusters, including several tumor and myeloid clusters, normal brain cells, T cells, endothelial cells, and mast cells. We observed a decrease in the number of proliferating tumor cells following TBK1i treatment, consistent with a role of TBK1 in cell proliferation. Conversely, TBK1i treatment led to an increase in mast cells. Interestingly, while we did not observe a significant change in T cell abundance within our 2-week dosing period, we noted an increase in CXCL9-producing cells, a cytokine crucial for T cell recruitment and activation. Additionally, receptor-ligand interaction analysis revealed a decrease in TAM-mediated SPP1 signaling. Together, these findings suggest a shift in the TME from a tumor-suppressive to an inflammatory state, providing a tentative mechanistic understanding of how TBK1 inhibition might synergize with anti-PD-1 treatment in GBM.

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

POSTER #43

IN VIVO CRISPR/CAS9 SCREENING IDENTIFIES FATTY ACID SYNTHASE (FASN) AS A TARGET TO ENHANCE ANTI-PD-1 THERAPY IN PANCREATIC CANCER

Feng Guo[1], Yaoqi Alan Wang[1]

[1] *Brown Center for Immunotherapy, Department of Medicine, Melvin and Bren Simon Comprehensive Cancer Center, Indiana University School of Medicine*

Email: *guof@iu.edu*

Purpose: PDAC exhibits significant resistance to anti-PD-1 immunotherapy, limiting its efficacy for the majority of patients. This study aimed to identify novel genetic regulators of immunotherapy resistance in PDAC using in vivo CRISPR/Cas9 screening. We sought to uncover actionable targets that could enhance the efficacy of anti-PD-1 therapy, while also elucidating the underlying mechanisms driving resistance.

Methods:A personalized sgRNA library was constructed by integrating bioinformatic analyses, including correlation with PD-1/PD-L1/MHC-I expression and immune scores derived from ESTIMATE, Library infected KPC cells were orthotopically implanted mice treated with anti-PD-1. Single-cell RNA sequencing (GEM-X technology) and flow cytometry were performed to evaluate immune microenvironment changes. The OVA-I model was employed to assess tumor cell-mediated activation of naïve CD8+ T cells and the cytotoxicity of activated CD8+ T cells in vitro. Different pancreatic tumor models were used to evaluate the therapeutic efficacy of FASN inhibition combined with anti-PD-1 therapy

Results

FASN was identified as one of the most depleted genes in the screen and the only top candidate with an inhibitor currently undergoing clinical trials. Knockout of FASN in KPC cell lines reduced tumor cell proliferation across multiple models and enhanced MHC-I expression, increasing antigen presentation. OT-I model demonstrated that FASN knockout improves the activation of naïve $CD8^+$ T cells and enhances $CD8^+$ T cells cytotoxicity against tumor cells.

Single-cell RNA sequencing of 41,000 CD45+ immune cells from tumors microenvironment revealed that FASN knockout had minimal impact on total T cell populations but led to a significant increase in cytotoxic T cells. Macrophage subpopulation analysis showed a decrease in precursor macrophages (monocyte-derived macrophages) and M2-like macrophages in the FASN knockout group. Combined transcriptomic analysis in tumor cell line indicated that FASN knockout suppressed CCL2 secretion, reducing monocyte recruitment and subsequent macrophage differentiation in the tumor microenvironment.

In multiple in vivo models, FASN knockout or inhibition combined with anti-PD-1 therapy significantly delayed tumor growth and extended overall survival in treated mice.

Conclusions:FASN inhibition enhances the efficacy of aPD-1 in PDAC through dual mechanisms. Tumor-intrinsic effects include reduced proliferation and increased antigen presentation, while microenvironmental effects involve T cell sensitization, reduced monocyte recruitment, and macrophage reprogramming. This partially shifts the immunosuppressive "cold" tumor microenvironment to a more inflamed "hot" state. These findings highlight the potential of combining FASN inhibitors with anti-PD-1 therapy to improve immunotherapy outcomes in PDAC.

This abstract has been selected for an oral presentation at the AACR Annual Meeting 2025 in Chicago (Minisymposium: Checkpoints and Modulators of Tumor Microenvironment, April 29, 2025).

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

POSTER #44

TARGETING REPLICATION PROTEIN A-DEPENDENT GAP PROTECTION IN BRCA-DEFICIENT AND PARP INHIBITOR RESISTANT CANCERS

Matthew Jordan[2], Pamela VanderVere-Carozza[2], Katherine Pawelczak[3], John Turchi[1,2]

[1] NERx Biosciences, Indianapolis, IN
[2] HEM/ONC
[3] NERx Biosciences

Email: *mattjord@iu.edu*

Two decades ago, the first synthetic lethal interaction for a cancer therapeutic agent was described with the use of poly (ADP)-ribose polymerase (PARP) inhibitors (PARPi) in BRCA-deficient cancers. The longstanding supposition was that PARPi-induced double strand breaks (DSBs) cannot be repaired by homologous recombination (HR) in BRCA-deficient cancer cells. However, recent work has highlighted an additional role for BRCA proteins and PARP in replication gap suppression; BRCA-deficient cells intrinsically possess single-stranded DNA (ssDNA) gaps that are exacerbated by PARPi. The major ssDNA binding protein replication protein A (RPA) is required to bind and protect these ssDNA gaps, and sufficient PARPi-induced ssDNA generation can exceed the RPA protection threshold resulting in DNA degradation, loss of genomic integrity, and cell death. A model was therefore posited that ssDNA gaps are the key chemotherapeutic lesions conferring sensitivity, rather than DSBs. Moreover, the model suggests therapeutic strategies to either induce more ssDNA to further exacerbate RPA protection, or to limit the protection capabilities of RPA would be viable. We have previously developed a small molecule RPA inhibitor (RPAi) NERx-329 that inhibits RPA ssDNA binding and exhibits anti-cancer activity as a single agent and in combination with DNA damaging agents or other DNA damage response (DDR) inhibitors. Notably, RPAi synergize with PARPi in BRCA1-deficient cells. To evaluate this mechanism of action, we performed DNA fiber combing assays and found that RPAi abolish the protection of PARPi-induced ssDNA gaps, resulting in DNA degradation of the replication forks. This degradation compromises genomic integrity and results in chromosome pulverization. ATR is the major DDR kinase that directs the cellular response to elevated levels of ssDNA, like that induced by PARPi, to ensure genomic stability by reducing replication stress and directing the S and G2-M checkpoints. ATR also depends upon RPA binding to ssDNA as a platform for activation, therefore we assessed the impact of RPAi on ATR activation using an *in vitro* reconstitution with purified proteins. We found that RPAi effectively inhibit ATR kinase activity and possess advantages over traditional ATRi that are ATP-competitive and exhibit reduced activity at physiological ATP concentrations. Taken together, RPAi promote DNA damage by inhibiting the ATR kinase and by abolishing the protection of PARPi-induced ssDNA gaps. As nearly all patients eventually develop PARPi resistance through a variety of mechanisms, we generated PARPi resistant cell lines from the BRCA1-deficient MDA-MB-436 TNBC cell line and found that RPAi remain potent inhibitors despite PARPi resistance. Collectively, this work characterizes a unifying RPAi mechanism for blocking ssDNA gap protection that can be utilized in numerous other gap-forming genetic backgrounds and offers a therapeutic strategy for systems of acquired resistance.

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

POSTER #45

REGULATION OF IRON METABOLISM BY HYPOXIA AND ITS IMPLICATION IN LEUKEMIA THERAPY

Lei Liu[2,3,4], Sankalp Srivastava[2,3,4], Ji Zhang[1]

[1] *Department of Pediatrics, Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN*

[2] *Department of Pediatrics*

[3] *Herman B Wells Center for Pediatric Research*

[4] *Indiana University School of Medicine*

Email: *lli5@iu.edu*

Acute lymphoblastic leukemia (ALL) is the most common blood cancer in children. It is an aggressive malignancy of the blood and bone marrow, characterized by the abnormal production of immature B- or T-lymphoblasts. It primarily affects children but can also occur in adults. Thanks to the advances in relevant research, ALL treatments have improved significantly, especially in pediatric patients, with 5-year survival rates exceeding 90% in some cases. However, challenges remain, like non-responding to initial therapy, drug resistance and relapsed/refractory leukemia, particularly in adult patients and certain subtypes of ALL. Leukemia stem cells (LSCs) are a subpopulation of leukemia cells characterized with self-renewal capabilities, which are thought to be the driving factors for the initiation, progression, therapy resistance and relapse encountered in ALL treatments. They frequently reside in a tumor microenvironment (TME) like bone marrow niches which contain a unique feature of low oxygen tension known as hypoxia. The hypoxic niches provide a favorable environment for LSCs, where they receive signals to maintain survival/self-renewal and promote resistance to conventional therapeutics. Therefore, it is essential to understand how leukemic cells respond to hypoxia, particularly metabolic vulnerability, which could facilitate the development of better therapeutic interventions to improve the outcomes in ALL patients. Dysregulation of iron metabolism has been observed in leukemia cells, and systematic iron accumulation is reported to be closely associated with the occurrence and progress of leukemia in patients. However, the regulation of iron metabolism by hypoxia in leukemic cells and its therapeutic implications have not been extensively explored. Here we show that the intracellular concentration of ferrous iron (Fe2+) was reduced in human ALL cells of DND-41 and RS4;11 cultured under hypoxic conditions (1% oxygen) comparing to those under ambient oxygen level. It indicates that iron metabolism is involved in the adaptive process of these cells to mitigate the stress of low oxygen tension. Furthermore, exogenous iron supplementation under hypoxic conditions induced significant cell death in multiple ALL cell lines while having little effect on cells cultured under normoxia, highlighting that disrupting iron homeostasis selectively kills leukemic cells cultured under hypoxia. Thus, we hypothesize that perturbation of iron metabolism in ALL cells under hypoxic conditions could compromise cellular adaptation to hypoxia to increase cell death and manipulating intracellular iron content may enhance the therapeutic outcomes when combined with chemotherapies. Further work is ongoing to explore the mechanism of iron regulation by hypoxia in ALL cells, and the potential to disrupt iron metabolism combined with chemotherapies to treat ALL *in vivo*.

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

POSTER #46

UNVEILING GENETIC PREDICTORS OF KU SENSITIVITY IN DNA DAMAGE RESPONSE.

Pamela L. Mendoza-Munoz[2], John J. Turchi[1]

[1] *Department of Medicine/ Indiana University/ School of Medicine, Indianapolis, IN*
[2] *Department of Medicine/ Indiana University/ School of Medicine*

Email: *pmendoza@iu.edu*

Background. The Ku 70/80 heterodimer, a key sensor of DNA double-strand breaks (DSB) is essential for DNA-PK activation, and the formation of the DNA-PK complex, playing a critical role in the non-homologous end joining (NHEJ) repair pathway. DNA-PK inhibition is a therapeutic strategy to modulate DSB repair pathways toward improving the clinical efficacy of ionizing radiation (IR) and DNA-damaging chemotherapies. We have developed Ku-DNA binding inhibitors (Ku-DBi), as a unique strategy of DNA-PK inhibition and demonstrated their potent and specific inhibitory activity. Ku-DBi showed varied efficacy across non-small cell lung cancer (NSCLC), and Triple negative breast cancer (TNBC) cell lines, differing from DNA-PK direct active site inhibition (NU7441). These observations suggest novel genetic predictors of sensitivity driven by DNA-PK-independent roles of Ku. **Methodology.** To explore the effects of specific mutations on cellular sensitivity to Ku inhibition, cellular viability assays were conducted in ATM knock-down NSCLC A549, BRCA1-deficient MDA-436, and wild type MDA-468 TNBC cell lines, as well as BRCA1 isogenic ovarian carcinoma UWB1.289 models. A genome-wide CRISPR/Cas9 KO screen was performed in NALM6 cells using the ChemoGenix platform (IRIC; University of Montreal, Canada) to uncover genes influencing cell proliferation in response to Ku-DBi treatment. A CRISPRi screen in NSCLC A549 cells was conducted to identify DDR genes driving cellular sensitivity to Ku inhibition. Ku-DBi's impact on tumor growth proliferation was assessed across 9 human tumor cancer panels (NCI-60, Developmental Therapeutics Program, DTP), with comparative analysis against existing DNA-PK inhibitors in the NCI database. **Results.** Our findings provide preliminary evidence on the identification of vulnerabilities to Ku inhibition mediated by Ku-DBi treatment. Sensitizer genes linked to mitochondrial metabolism were identified, whose KO is predicted to result in synthetic lethality interact to Ku-DBi. These inactivating mutations, associated with tumor development in multiple cancers, align with the strong responses observed as potent growth inhibition greater than 70% in breast, colon, renal, and ovarian cancers in the NCI screen, and high lethality exceeding 70% in five leukemia cell lines. In this context, NHEJ is crucial for maintaining genomic stability during T cell development, while Ku plays a key role in supporting B cell survival and proliferation through its DNA repair functions. **Conclusions.** These findings represent a significant advancement in the characterization of Ku-DBi and provide a basis for further genetic and pharmacologic validation, aiding patient selection and the exploration of single-agent and combination therapies.

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

POSTER #47

PROTEIN ARGININE METHYLTRANSFERASES 1 (PRMT1) AS A THERAPEUTIC POTENTIAL TARGET FOR TREATMENT OF MULTIPLE MYELOMA

HONG PHUONG NGUYEN[1], Enze Liu[2], Brian Walker[2], Ngoc Tung Tran[1]

[1] Well Center for Pediatric Research, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, USA
[2] Melvin and Bren Simon Comprehensive Cancer Center, Division of Hematology and Oncology, School of Medicine, Indiana University, Indianapolis, IN, USA

Email: *nguyeho@iu.edu*

Arginine methylation, catalyzed by protein arginine methyltransferases (PRMTs), is a key post-translational modification involved in transcription, RNA splicing, signal transduction, metabolism, and protein homeostasis. PRMT1, a Type I PRMT enzyme, is responsible for most asymmetric di-methylation in cells and is linked to poor survival in solid and hematological cancers. However, its role in multiple myeloma (MM) remains unclear.

MM is a plasma cell malignancy characterized by uncontrolled plasma cell expansion in the bone marrow, leading to hypercalcemia, renal failure, anemia, and bone lesions. Despite therapeutic advancements, relapsed/refractory (RR) MM remains incurable, necessitating novel treatment targets.

Transcriptomic analysis revealed PRMT1 as the highest expressed PRMT in MM cell lines and patient samples, with elevated expression in relapsed cases and a strong correlation with poor prognosis. We hypothesized that PRMT1 is essential for MM survival. Using CRISPR/Cas9, we found that PRMT1 deletion significantly inhibited MM cell growth and induced apoptosis. Pharmacological inhibition of PRMT1 with MS023 led to cell cycle arrest and cell death in both newly diagnosed and RR MM patient samples. In vivo studies, genetic and pharmacologic PRMT1 suppression robustly reduced tumor growth and burden.

These findings demonstrate PRMT1 as a critical regulator of MM, presenting a promising therapeutic target Ongoing studies aim to elucidate PRMT1's mechanisms in MM and assess its therapeutic potential alone or in combination with standard-of-care treatments. Our prelimiary mechanistic studies will also be presenting in this story

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

POSTER #48

THE OLIGOSACCHARYLTRANSFERASE (OST) COMPLEX IS A CRITICAL REGULATOR OF MULTIPLE MYELOMA PLASMA CELL FUNCTION

HONG PHUONG NGUYEN, Ngoc Tung Tran, Enze Liu, Brian A Walker, Anh Quynh LE, Mahesh Lamsal, Harikrishnan Hemavathy

Email: *nguyeho@iu.edu*

Multiple myeloma (MM) is an incurable malignancy driven by the clonal expansion of mutated plasma cells in the bone marrow, leading to severe clinical complications. Identifying new therapeutic targets is essential for improving MM treatment. Here, we identify the oligosaccharyltransferase (OST) complex as a critical vulnerability in MM. Elevated OST complex expression correlates with high-risk, relapsed MM and poor prognosis. Targeting the OST complex impairs MM cell growth, induces cell cycle arrest and apoptosis, and enhances sensitivity to bortezomib in vitro and in vivo by suppressing genes linked to bortezomib resistance. Mechanistically, OST inhibition downregulates key pathological pathways in MM, including mTORC1 signaling, glycolysis, MYC targets, and cell cycle regulation, while promoting TRAIL-mediated apoptosis. Notably, MYC translation is significantly reduced upon OST complex inhibition. These findings establish the OST complex as a promising therapeutic target in MM and suggest that its inhibition, in combination with bortezomib, could be an effective strategy for relapsed MM patients.

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

POSTER #49

TRANSLATIONAL REGULATION OF MYC IN ACUTE LYMPHOBLASTIC LEUKEMIA

Sankalp Srivastava[1], Jagannath Misra[2], Rodney Claude[2], Michael Holmes[2], Gang Peng[2], Kirk Staschke[2], Sandeep Batra[2], Utpal Dave[2], Chi Zhang[2], Ronald Wek[2], Jing Fan[3], Ji Zhang[2]

[1] Department of Pediatrics, IU School of Medicine, Indiana University
[2] IU School of Medicine, Indiana University
[3] Morgridge Institute for Research, University of Wisconsin-Madison

Email: *sasriv@iu.edu*

Acute lymphoblastic leukemia (ALL) is the most frequently diagnosed childhood cancer, with a prevalence of almost 25%. ALL cells are auxotrophic for asparagine and have been targeted therapeutically by treatment with bacterial-derived asparaginase. Although asparaginase has been a cornerstone of treatment, ALL can relapse in 10-15% of cases. We previously published that asparagine synthetase (*ASNS*) induction in response to asparagine depletion is a primary resistance mechanism to asparaginase therapy. Using auxotrophic DND-41 cells, we also showed that asparagine starvation suppresses the expression of c-MYC (MYC) protein, a critical oncogenic driver in ALL cells. We did not observe a change in *MYC* transcript levels or protein stability, suggesting the acute decrease in protein level was due to a decrease in translation of *MYC* mRNA. We performed a polysome profiling in DND-41 cells to measure the translation of *MYC* mRNA. Compared to asparagine-replete control, asparagine starvation caused a significant decrease in the amount of heavier polysomes associated with *MYC* mRNA. We further performed a Ribo-seq experiment, where cell lysates were treated with RNAse I, leaving ribosome-protected fragments (RPFs) intact for high throughput sequencing. Our analysis showed that RPFs were distributed throughout the *MYC* coding sequence (CDS) for the control samples but enriched in the N-terminal of the *MYC* CDS when asparagine was limiting. Of note, these RPFs were around closely spaced asparagine codons (+4, +9, +11) near the translation initiating AUG codon, suggesting this is a critical cis-element regulating the translation of *MYC* mRNA. We designed a MYC-translation reporter with the MYC N-terminal region fused to an eYFP-DD construct. The fused protein rapidly degrades upon translation due to the destabilization domain (DD), leading to reduced fluorescence. Treating cells with a small molecule, trimethoprim (TMP), stabilizes the fusion protein, enhancing fluorescence. We defined the translation rate by calculating the ratio of fluorescence intensities with and without TMP. Our results showed that fusing the MYC N-terminal region to the reporter significantly reduced translation compared to the eYFP-DD construct upon asparagine starvation. We also found that deleting the asparagine codons partially rescued the reporter translation, when asparagine is limiting. We will use this system to identify trans-factors regulating *MYC* translation using a dedicated CRISPR library targeting mRNA regulators.

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

POSTER #50

EXPRESSION OF ICAM-1 IN SPECTRUM OF ORAL SQUAMOUS CELL CARCINOMA

Sofia Ali Syed[2], Paige Sylvester[1], Carson Walton[1]

[1] *Department of Otolaryngology- Head & Neck Surgery, Indiana University School of Medicine, Indianapolis, IN*
[2] *Department of Otolaryngology- Head & Neck Surgery, Indiana University School of Medicine*

Email: *sofisyed@iu.edu*

Background:

Oral squamous cell carcinoma (OSCC) is a prevalent head and neck cancers, representing a significant global health burden due to its high incidence, aggressive nature, and poor survival rates despite advancements in cancer treatment, as recurrence and late-stage diagnosis remain critical challenges with a poor survival rate and limited therapeutic options. Understanding the molecular mechanisms driving OSCC progression is essential for identifying novel biomarkers and therapeutic targets.Intercellular adhesion molecule 1 (ICAM-1), a transmembrane glycoprotein involved in cell adhesion and signal transduction, plays a key role in various pathologic conditions, including inflammation, immune response, and cancer progression. While ICAM-1 has been implicated in promoting tumor growth, invasion, and metastasis in several malignancies, its role in oral carcinogenesis remains underexplored. This study aimed to investigate ICAM-1 expression in OSCC and its potential role in tumorigenesis.

Methods:

We employed a 4-NQO-induced oral carcinogenesis mouse model to simulate the progression of OSCC. Tissue samples representing different stages of oral carcinogenesis—including hyperplasia, dysplasia, papillomas, and invasive carcinoma—were collected and processed into paraffin-embedded sections. Immunofluorescence staining was performed to analyze ICAM-1 expression across these stages.

Results:

We successfully established a 4-NQO-induced oral carcinogenesis mouse model that recapitulates the progressive stages of oral cancer, including hyperplasia, dysplasia, papillomas, and invasive carcinoma. The intact oral epithelium is ICAM-1 negative, and its expression was first observed in hyperplastic tissues, where 87.5% of samples were positive, while 12.5% showed no expression. In the subsequent stages of carcinogenesis—mild to moderate dysplasia, severe dysplasia or carcinoma in situ, and papillomas—ICAM-1 was consistently expressed in 100% of tissues. Overall, ICAM-1 was expressed in 93.2% of OSCC tissues, with only 6.8% showing no detectable expression. These findings demonstrate that ICAM-1 expression is highly prevalent in oral cancer tissues.

Conclusions:

ICAM-1 expression progressively increases during oral carcinogenesis, with high prevalence in OSCC tissues. Its early upregulation in hyperplasia and consistent expression in advanced stages suggests a potential role in promoting tumor initiation and progression. These findings highlight ICAM-1 as a promising biomarker for early OSCC detection and a potential therapeutic target for combating tumor progression and metastasis.

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

POSTER #51

THE PHARMACOGENOMIC AND IMMUNE LANDSCAPE OF SNORNAS IN HUMAN CANCERS

Runhao Wang[1,2,3], Chengxuan Chen[1,2,3], Yuan Liu[1,2,3]

[1] *Biostatistics and Health Data Science*
[2] *Brown Center for Immunotherapy*
[3] *Indiana University School of Medicine*

Email: *wangrunh@iu.edu*

Small nucleolar RNAs (snoRNAs) are a class of non-coding RNAs primarily known for their role in the chemical modification of other RNAs. Recent studies suggested that snoRNAs may play a broader role in anti-cancer treatments such as targeted therapies and immunotherapies. Despite these insights, the comprehensive landscape of snoRNA associations with drug response and immunotherapy outcomes remains unexplored. In this study, we identified 79,448 and 75,185 associations between snoRNAs and drug response using data from VAEN and CancerRxTissue, respectively. Additionally, we discovered 29,199 associations between snoRNAs and immune checkpoint genes and 47,194 associations between snoRNAs and immune cell infiltrations. Sixteen snoRNAs were significantly correlated with immunotherapy objective response rate (ORR), and 92 snoRNAs showed significantly differential expression between cancers with high and low ORR. Furthermore, we identified 17 snoRNAs with significantly differential expression between cancer types with high and low immune-related adverse event(irAE) reporting odds ratio (ROR). To facilitate further research, we developed a user-friendly portal, Pharmacogenomic and Immune Landscape of SnoRNA (PISNO, https://hanlaboratory.com/PISNO/), enabling researchers to visualize, browse, and download multi-dimensional data. This study highlights the potential of snoRNAs as biomarkers or therapeutic targets, paving the way for more effective and personalized anti-cancer treatments.

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

POSTER #52

ANCESTRAL DIFFERENCES IN ANTI-CANCER TREATMENT EFFICACY AND THEIR UNDERLYING GENOMIC AND MOLECULAR ALTERATIONS

Jingwen Yang[1,2,3], Mei Luo[1,2,3], Alejandro Schaffer[4], Chengxuan Chen[1,2,3], Yuan Liu[1,2,3], Yamei Chen[1,2,3], Chunru Lin[5], Lixia Diao[6], Yong Zang[2,3], Yanyan Lou[7], Huda Salman[1,8], Gordon Mills[9], Eytan Ruppin[4], Leng Han[1,2,3]

[1] *Brown Center for Immunotherapy, School of Medicine, Indiana University*
[2] *Department of Biostatistics and Health Data Science, School of Medicine, Indiana University*
[3] *Center for Computational Biology and Bioinformatics, Indiana University School of Medicine*
[4] *Cancer Data Science Laboratory, Center for Cancer Research, National Cancer Institute (NCI), National Institutes of Health (NIH)*
[5] *Department of Molecular and Cellular Oncology, the University of Texas MD Anderson Cancer Center*
[6] *Department of Bioinformatics and Computational Biology, the University of Texas MD Anderson Cancer Center*
[7] *Division of Hematology and Oncology, Mayo Clinic*
[8] *Division of Hematology-Oncology, Indiana University School of Medicine*
[9] *Knight Cancer Institute, Oregon Health and Science University*

Email: *jy82@iu.edu*

Previous systematic multi-omics analysis revealed ancestry-dependent molecular alterations; however, their impact on the efficacy of anti-cancer treatments remains largely unknown. In our study, we analyzed clinical trials from ClinicalTrials.gov and found that only 8,779 out of 102,721 (8.5%) oncology clinical trials provided information on enrollment by race/ethnicity. The underrepresentation of non-White populations indicates a significant challenge in determining differences in the efficacy of anti-tumor treatments across racial groups. Through a comprehensive analysis of clinically actionable genes (CAGs), imputed drug responses, and immune features across 24 cancer types from The Cancer Genome Atlas (TCGA), we identified potential differences in treatment response to targeted, chemo and immunotherapies between different ancestral populations. Further analysis of multiple independent cohorts, including multi-omics dataset and clinical trials, confirmed some of our key findings. These findings are made publicly available in a comprehensive web portal, Ancestral Differences of Efficacy in Cancers (ADEC; https://hanlaboratory.com/ADEC), to facilitate further investigation. Our study provides a global overview of ancestry-associated differences in therapeutic efficacy, highlighting the importance of considering ancestry in anti-cancer therapies.

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

POSTER #53

ELUCIDATE THE MOLECULAR MECHANISMS IN GASTRIC CANCER WITH PERITONEAL METASTASIS

Tadahito Yasuda[2], Y Alan Wang[1]

[1] *Department of Medicine, Brown Center for Immunotherapy, Indiana University School of Medicine, , IN*
[2] *Department of Medicine, Brown Center for Immunotherapy, Indiana University School of Medicine*

Email: *tayasuda@iu.edu*

Gastric cancer (GC) is a highly lethal disease, ranking as the fifth most frequently diagnosed cancer globally, with nearly one-third of patients presenting with peritoneal metastasis at diagnosis. However, the molecular mechanisms driving this malignancy which has a low response rate to conventional therapies remain unclear. This low response is likely due to the heterogeneity and immunosuppressive nature of the tumor microenvironment (TME), which we had reviewed in this subject area in two review articles recently (*Yasuda and Wang, Trends in Cancer, 2024; British Journal of Cancer, 2025*). Among the TME components, tumor-associated macrophages (TAMs) are known to exhibit significant heterogeneity in cancers, including GC. It is not yet clear which TAM subtypes promote the formation of peritoneal dissemination, but it is presumed that the formation of a niche during distal dissemination is crucial. Our preliminary data of single mass cytometry identified a subset of macrophages exhibiting senescence-associated secretory phenotype (SASP) in GC patients with peritoneal metastasis. Moreover, we have developed and validated the metastatic potential of a GC cell line, GAN-KPC (G12V-mutant *Kras/p53KO/Cdh1KO)* derived from gastric neoplasia mice (GAN) through gastric wall transplantation in C57BL/6 mice. Using this unique mouse model, we validated senescent TAM subset in distal metastatic sites. Furthermore, we have identified GC peritoneal metastatic niche and discovered that macrophages in the metastatic niche display a peculiar phenotype similar to neutrophils extracellular traps (NETs), before shifting to a senescent status. Based on my preliminary data, I hypothesize that subset of macrophages form NETs-like structure at peritoneal premetastatic niche and subsequently interact with cancer cells leading to senescent macrophages and formation and expansion of a robust niche. I plan to perform comprehensive analyses, including 10X Visium HD Spatial Transcriptomics, to further elucidate the significance of senescent macrophage subtype in the metastatic niche.

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

POSTER #54

MODULATING NLRP3 INFLAMMATION AND ION CHANNELS TO DISRUPT GBM PROGRESSION

I-Ju Yeh[1], Fletcher White[1]

[1] *Anesthesia*

Email: *ijuyeh@iu.edu*

CENTRAL HYPOTHESIS: Glioblastoma (GBM) is an aggressive brain cancer characterized by high invasiveness, resistance to therapy, and poor prognosis. The tumor microenvironment (TME), shaped by activated immune cells like microglia, plays a critical role in GBM progression. Microglia, the CNS's resident immune cells, become activated in GBM, adopting pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes that influence tumor progression and therapy resistance. A key pathway in microglial activation is the NLRP3 inflammasome, which produces pro-inflammatory cytokines like IL-1β and IL-18. Chronic inflammation within the TME, mediated by the activation of the NLRP3 inflammasome and ion channels, supports tumor growth, immune evasion, and therapy resistance. The NLRP3 inflammasome, activated by damage-associated molecular patterns (DAMPs), triggers the release of pro-inflammatory cytokines (IL-1β, IL-18), exacerbating inflammation and sustaining a feedback loop that promotes tumor survival and invasiveness. Ion channels, particularly potassium and sodium channels, modulate microglial activation and inflammation, and their dysfunction can further enhance this chronic inflammatory state. We seek to understand the mechanisms underlying chronic inflammation in the GBM TME, with a focus on ion channel modulation and inflammasome activation. By investigating these pathways, we aim to identify novel therapeutic strategies to disrupt the inflammatory cycle that drives GBM progression. Our central hypothesis is: *NLRP3 activation in TAMs sustains chronic inflammation in the glioblastoma TME, promoting tumor growth and immune evasion via ion channel modulation*.

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

POSTER #55

EXPRESSION PROTEOMICS OF LIVER FIBROSIS IN HEPATIC STELLATE CELLS THROUGH OLINK WORKFLOW AND MASS SPECTROMETRY.

Ian Green[1,6], James Rooney[2], Mckayla Stevens[3], Whitney Smith-Kinnaman[1], Jessica Maiers[4], Amber Mosley[5]

[1] *IU Simon Comprehensive Cancer Center, Indianapolis, IN*
[2] *Department of Biochemistry and Molecular Biology, Indianapolis, IN*
[3] *IU Simon Comprehensive Cancer Center*
[4] *Department of Medicine, Indianapolis, IN*
[5] *Department of Biochemistry and Molecular Biology, Department of Medicine, Center for Proteome Analysis, Indianapolis, IN*
[6] *Department of Biochemistry and Molecular Biology*

Email: *itgreen@iu.edu*

Liver disease is on the rise with 1 in 400 adults in the U.S. estimated to have cirrhosis. Cirrhosis is a disease characterized bypersistent scarring of the liver, termed fibrosis, in response to chronic insult. Advancement of fibrosis to cirrhosis is irreversible and comes with an increased risk of cancer development. Tolimit the development of cirrhosis, studying fibrosis and the underlying proteins promoting it is of great importance. Hepatic stellate cells (HSC) are the primary cells contributing to fibrosis, producingfibrogenic factors and extracellular matrix proteins encouraging the initiation and propagation of fibrosis. In our study, we soughtto analyze the intracellular proteins driving fibrogenesis in HSCs. To achieve this, we are using two proteomic methods, discovery proteomics via mass spectrometry (MS), and affinity proteomics via Olink workflow. In addition to two proteomic methods two data types are produced, abundance data and thermal stability data. Thermal stability data is unique in that it measures a change in the activity of the protein. To achieve this, protein samples are treated at a range of temperatures and the soluble proteins are integrated together.Any change in protein activity may change the temperature at which it melts, impacting its solubility and subsequent detection. To identifyprotein changes during fibrogenesis we treated primary human HSC with the profibrotic cytokine TGFβ for 8 and 24 hours paired with a vehicle control, extracted the intracellular proteins, and prepared samples for abundance and thermal stability analysis. The sets of samples were analyzed via Tandem-MS and Olinkproducing expression proteomics data. The significant proteins (p-value<0.05) werevalidated in immortalized HSCs (LX-2 cells) and analyzed for novelty and relation to fibrosis. These tasks were performed by using resources such asDepMap, PubMed, Ingenuity Pathway Analysis,Uniprot, Reactome, and Gene Ontology. From these analyses, we selected proteins todetermine their importance to fibrogenesis. LX-2, an immortalized human hepatic stellate cell line,were infected with lentiviruses engineered to express shRNA targeting TGFBI, SERPIA1, DNAJB11, and PALLD. We are currently analyzing these cells toobserve the effects on the abundance of key fibrotic markers such as α-SMA, fibronectin, and collagen in response to TGFβ.Future work includes the normalization of thermal stability data produced byOlink Thermal stability data from PISA is asstated earlier, is the accumulation of protein across and increasing temperature gradient. One limitation of this is the increase in abundance of proteins is not accounted for in standard PISA data. To remedy this PISA data is normalized using abundance data, which outputs a normalized thermal stability reading. To achieve this a code is being produced in R to normalize PISA data produced by Olink.

Basic Science ***Post-baccalaureate***

POSTER #56

EXAMINING EXTRINSIC PHYSICAL INFLUENCES ON HEMATOPOIETIC STEM CELL EXPANSION

Wendy Deras[3], Lindsay Wathen[1], Melissa Kacena[2], Milos Marinkovic[2], Maegan Capitano[1]

[1] *Department of Microbiology & Immunology,Indiana University School of Medicine, Indianapolis, IN*
[2] *Department of Orthopaedic Surgery, Indiana University School of Medicine, Indianapolis, IN*
[3] *Department of Microbiology & Immunology,Indiana University School of Medicine*

Email: *wmderas@iu.edu*

Degradation of bone and increased incidence of blood disorders in the aging population often occur together, leading to various health outcomes and imposing a significant economic burden. The decline in osteogenic and hematopoietic function within the bone marrow (BM) of aging populations is associated with reduced bone mineral density, impaired healing, heightened risks of hematological malignancies, and worsening conditions such as anemia, neutropenia, and thrombocytopenia. The BM niche is managed by an intricate regulatory network which include mesenchymal stem cells (MSCs), cytokine/growth factors, hematopoietic stem and progenitor cells (HSPCs), mature immune cells, and the extracellular matrix (ECM). The ECM is essential for the regulation of hematopoiesis (the process by which blood cells are formed). With aging, the equilibrium of the regulatory network shifts. The transition in aged MSCs from bone-forming (osteogenic) to fat-forming (adipogenic) development is associated with the tendency of HSPCs– responsible for generating blood and immune cells – to shift towards the myeloid lineage. However, the biological mechanism through which the aging BM niche, particularly the interactions between HSPCs, MSCs, and the ECM, contributes to hematopoietic dysfunction with age remains unclear. Building on advancements in ECM biology, this study sought to clarify how age-related changes in the ECM and to MSC regulatory cues affect HSPC function. Using specialized biofabricated plates, which had wells coated with microphysiological analogues of young (≤ 25 years old) or aged (≥ 60 years old) BM ECMs, created by human MSCs, I explored how the ECM influences HSPC development. Utilizing HSPCs isolated from cord blood units, I evaluated HSPC function using colony-forming unit assays and characterized them immunophenotypically through flow cytometry at input and following 7 days of culture on either tissue culture-treated (TCP), young ECM (yECM), or aged ECM (aECM) wells. I demonstrated that all conditions resulted in increased nucleated cell number compared to input after expansion. By phenotype, we saw: 1) significant increases of HSCs and Multipotent Progenitors (MPPs) compared to input only when cultured on aECM, 2) significant increases in Multi-lymphoid Progenitors (MLPs) in all culture conditions when compared to input, 3) significant increases in Common Myeloid Progenitors (CMPs) when cultured on either ECM condition, and 4) no changes to Granulocyte-Monocyte Progenitor (GMP) numbers in any condition. There were no functional differences demonstrated by colony formation other than those associated with an increased proportion of those cells in culture. This suggests that perhaps different culture conditions favor the expansion of different cell populations within less 'mature' cord blood HSPCs. In the future, we wish to examine the effect of ECM on 1) a more 'mature' HSPC population (isolated from BM), and 2) a co-culture system of HSPCs and MSCs on our ECM plates to examine the crosstalk between these two populations.

Basic Science ***Post-baccalaureate research fellow***

POSTER #57

CRISPR-MEDIATED KNOCKDOWN OF PTPN2 PROMOTES THE EXPRESSION OF IL-9 IN HUMAN T CELLS

Kent Williams[1,4], Sanam Benam[2], Jiazhi Xu[3], Reza Shahbazi[2], Lionel Apetoh[3]

[1] *IU Melvin and Bren Simon Comprehensive Cancer, Indianapolis, IN*
[2] *Department of Hematology and Oncology, Indianapolis, IN*
[3] *Brown Center for Immunotherapy, Department of Microbiology and Immunology, Indianapolis, IN*
[4] *Brown Center for Immunotherapy*

Email: *kew5@iu.edu*

The presence of Interleukin 9 (IL-9) has been demonstrated to promote immune-mediated anti-tumor response in multiple cancers. It is hypothesized that IL-9 expression is driven by activation of JAK/STAT signaling. Such signaling pathways are tightly controlled through phosphorylation and dephosphorylation by enzymes such as the tyrosine-protein phosphatase non-receptor type 2 (PTPN2). To better understand how PTPN2 affects cytokine expression in IL-9-secreting CD4 (Th9) and CD8 (Tc9) cells, PTPN2 was knocked down in human T cells via CRISPR and those cells were differentiated into Th9 and Tc9. Following differentiation, cells were stimulated with PMA/Ionomycin and placed into chips for single-cell secretome analysis using the Isocode Human Adaptive Immune System kit for the IsoSpark by Bruker. This enabled the analysis of IL-9 secretion in addition to the secretion of 31 other cytokines, with some cells secreting more than one (polyfunctional). PTPN2 KD increased the total number of Th9 cells but not Tc9 cells relative to controls, but increased the overall expression of IL-9 within both subsets. PTPN2 KD in Th9 also increased the number of certain polyfunctional groups, driven largely by increased TNFα expression. This study demonstrates the importance of PTPN2 in the regulation of IL-9 expression while highlighting the usefulness of the IsoSpark in evaluating the secretomic profiles of immune cells.

Basic Science ***Research Analyst***

POSTER #58

IDENTIFYING FKBP5 AS A NOVEL MEDIATOR OF CANCER CACHEXIA

Felipe Cardona Polo[1,2]

[1] *Indiana University School of Medicine*
[2] *Anatomy, Cell Biology and Physiology Department*

Email: *lfcardon@iu.edu*

Background: Cancer is frequently accompanied by cachexia, an uncured multi-organ wasting syndrome that leads to impaired musculoskeletal health, physical function, and overall survival. FK506-binding protein-51 (FKBP5) is a co-chaperone and mediator of the stress response, with recent findings suggesting a regulatory role of FKBP5 in metabolic disease. Here, we investigated FKBP5's role in the development of cancer cachexia. **Methods:** Expression of skeletal muscle FKBP5 was assessed in tumor-bearing and control mice. Cultured C2C12 myotubes were overexpressed with FKBP5, or FKBP5 was silenced in myotubes exposed to tumor cells. 10-week-old male wildtype (WT) C57BL/6J or FKBP5$^{-/-}$ (KO) mice (n=6-12/group) received intrasplenic injections with MC38 colorectal cancer cells ($1.25x10^5$) to induce liver metastases (LM), while controls received saline (Sham). Animals were assessed for indices of cachexia including muscle mass, torque, and bone quality. **Results:** Expression of FKBP5 was significantly increased ($p<0.05$) in skeletal muscle of mice bearing lung (LLC), pancreatic (KPC), and colorectal (C26, HCT116, MC38) cancers. *In vitro* studies demonstrated that FKBP5 overexpression caused myotube atrophy (-26%; $p<0.0001$) and reductions in AKT/mTOR, while genetic blockade of FKBP5 protected against cancer-induced myotube atrophy (+20%; $P<0.0001$). WT-MC38 hosts displayed reductions in muscle mass (quadriceps: -25%; gastrocnemius: -24%; tibialis anterior: -20%; $p<0.0001$) and plantarflexion torque ($p<0.01$) compared to WT-Sham. Meanwhile, muscle mass and torque were unchanged in KO-MC38 compared to KO-Sham. Supporting the phenotype, we observed significant upregulation of murf1 and atrogin1 in the skeletal muscle of WT-MC38, which was counteracted in KO-MC38. Like muscle, cortical bone volume fraction (Ct.BV/TV: -9%) and trabecular bone volume fraction (Tb.BV/TV: -33%) were reduced in WT-MC38 compared to WT-Sham, while differences were not observed between KO-MC38 and KO-Sham. **Conclusion**: Our data suggests that targeting FKBP5 protects against cancer cachexia, representing a new strategy to improve musculoskeletal health and quality of life in cancer patients.

Basic Science ***Research Technician***

POSTER #59

HUMAN LUNG-DERIVED EXOSOMES ENHANCE STEMNESS AND LONG-TERM ENGRAFTMENT POTENTIAL OF HEMATOPOIETIC STEM CELLS

Sanam Rezaei Benam[1], Xuepeng Wang[2], Samaneh Maleknia[1], Reuben Kapur[2], Reza Shahbazi[1]

[1] *Division of Hematology/Oncology, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN*
[2] *Herman B Wells Center for Pediatric Research, Indiana University, Indianapolis, IN*

Email: *srezaeib@iu.edu*

Long-term hematopoietic stem cells (LT-HSCs) are essential for the continuous regeneration of blood and immune cells throughout life. Despite their clinical significance in bone marrow transplantation, the extrinsic factors modulating LT-HSC self-renewal and engraftment remain poorly understood. Our study investigates the role of exosomes derived from human lung epithelial cells in enhancing LT-HSC function and explores the underlying molecular mechanisms using multiomics analyses. Exosomes were isolated from human small airway epithelial cells (P. Lung) and other cell types and characterized using size exclusion chromatography, flow cytometry, and electron microscopy. Labeled exosomes were incubated with purified CD34+ human HSCs, and uptake was analyzed via confocal microscopy, flow cytometry, and ImageStream. Functional assays including colony-forming unit (CFU) tests and xenotransplantation in immunodeficient mice were performed. Multiomics analyses—single-cell RNA-seq (CITE-seq), proteomics, lipidomics, and miRNA sequencing—were used to identify gene regulatory networks influenced by exosomes. Lung-derived exosomes showed highly specific uptake by LT-HSCs, with minimal interaction with progenitor populations. Exosome treatment significantly increased CFU numbers and multilineage colonies. In vivo, both primary and secondary xenograft studies demonstrated enhanced human CD45+ cell engraftment in exosome-treated groups, with increased T cell and myeloid differentiation. Multiomics analysis revealed expansion of LT-HSC

subpopulations in bone marrow and peripheral blood and identified key differentially expressed genes, including upregulation of S100A9, MS4A6A, and RUNX2, and downregulation of HOMEZ and CD69. Exosomal payloads contained stemness-associated proteins (e.g., ALDH2, JAK1, STAT1) and regulatory miRNAs (e.g., miR-146a-5p, miR-200c-3p, miR-455-3p). Lipidomics revealed an abundance of sphingomyelins and phosphatidylcholines, suggesting a role in exosomal stability and cellular interaction. Notably, exosomes suppressed inflammatory and stress response gene programs in LT-HSCs, favoring quiescence and self-renewal. Human lung-derived exosomes selectively target LT-HSCs, enhance their stemness and engraftment capacity, and modulate gene expression through coordinated delivery of proteins, lipids, and miRNAs. These findings uncover a novel extrinsic regulatory mechanism in hematopoiesis and highlight the potential of exosome-based therapies to improve outcomes in hematopoietic stem cell transplantation and treatment of hematological disorders.

Basic Science ***Research Technician***

POSTER #60

IRON ACCUMULATION CORRELATES WITH TUMOR PROGRESSION IN MOUSE MODELS OF PROSTATE CANCER

Jennifer Rooks[1], Erin Perkins[1], Caleb Taylor[1], Asmaa Elkenawi[1]

[1] Department of Urology, Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indiana University School of Medicine

Email: *jnrooks@iu.edu*

Tumor-associated macrophages (TAMs) are well-known as anti-inflammatory immune cells that contribute to various facets of prostate cancer development. An emerging aspect of TAMs is their "iron-rich" phenotype. This accumulation of iron can contribute to cancer cell initiation and tumor progression. In the current study, we aim to explore the key role of TAMs in regulating iron balance within the prostate tumor microenvironment (TME). We induced prostate tumorigenesis in the NP mice using Tamoxifen. The NP mice (*Nkx3.1*$^{CreERT2/+}$ *; Pten*$^{flox/flox}$ *; Rosa26-CAG-LSL-EYFP/+* mice) develop prostate intraepithelial neoplasia and localized prostate adenocarcinoma by 12 months of age. We also utilized male TRAMP which spontaneously develops poorly differentiated prostate tumors reminiscent of the neuroendocrine subtype. We then stained prostate tissues using different immune cell markers and Perls''s method which stains "non-heme" iron to identify cells with high abundance of iron. Our results demonstrate that prostate tissues from two distinct transgenic prostate cancer models (adenocarcinoma and neuroendocrine), spanning different developmental stages (12 and 18 months) exhibited high level of non-heme iron in stromal regions compared with non-tumor bearing controls. The areas with high iron abundance exhibited high expression of F4/80 and the iron exporter Ferroportin-1, also known as solute carrier family 40 member 1 (SLC40A1). In summary, we found that the availability of non-heme iron correlates with the abundance of TAMs in prostate tumor microenvironment. Future studies will focus on understanding how TAMs are regulating iron availability in the dynamic tumor environment.

Basic Science ***Research Technician***

POSTER #61

TRANSIENT DELETION OF RAPSYN ALTERS NEUROMUSCULAR FUNCTION

Jing Xu

Email: *jxu11@iu.edu*

Background: Cancer patients often develop cachexia, a chronic wasting disease characterized by the loss of skeletal muscle mass and function. Cachexia is responsible for up to 30% of all cancer-related deaths, making early detection and treatment a priority. Previous investigations have found reduced levels of skeletal muscle Rapsyn in cachectic mice. Rapsyn, a postsynaptic scaffolding protein, plays a critical role in acetylcholine receptor clustering and stability of the NMJ. Prior evidence has shown impaired NMJ development in Rapsyn-deficient mice. However, these animals are not viable after birth—impeding investigations on the Rapsyn-dependent effects in adult skeletal muscle. Here, we used an inducible Cre-ERT2 system to determine the effects of silencing Rapsyn on skeletal muscle. Methods: Adult rapsnfl/fl and rapsnfl/fl-HSA Cre mice were injected with tamoxifen for 5 days (n=5/group). Following a 14-day washout period, animals were assessed for indices of neuromuscular function and skeletal muscles were collected for further analyses. Results: Short-term deletion of skeletal muscle Rapsyn did not exert significant effects on body composition, or lead to overt atrophy of quadriceps, tibialis anterior, or gastrocnemius muscles. In contrast, Rapsyn deletion increased single motor unit potentials (+65.1%, $p<0.01$), reduced motor unit number estimation (-68.1%, $p<0.05$), and caused reductions in normalized plantarflexion torque (-8.00%, $p<0.05$), suggesting impaired neuromuscular function. Follow-up molecular analyses revealed lower gene expression of musk, while staining for neural cell adhesion molecule (NCAM) in the tibialis anterior muscles revealed a significant increase in the percentage of NCAM positive fibers. Conclusion: Our data suggest that acute deletion of skeletal musclerapsn initiates dysregulation of neuromuscular function and alters NMJ homeostasis.

Basic Science ***Research Technician***

POSTER #62

INVESTIGATING NOVEL NUCLEAR RECEPTOR BINDING MOTIFS IN HEAT SHOCK FACTOR 1 AND ITS INTERACTION WITH ESTROGEN-RELATED RECEPTOR α IN BREAST CANCER

David Adelfinsky[4], Sunandan Chakrabarti[1], Yuan Feng[2], Jason Tennessen[2], Richard Carpenter[3]

[1] *Medical Sciences, Indiana University School of Medicine-Bloomington, Bloomington, IN 47405, Bloomington, IN, Bloomington, IN*

[2] *Department of Biology, Indiana University-Bloomington, Bloomington, IN 47405, Bloomington, IN, Bloomington, IN*

[3] *Medical Sciences, Indiana University School of Medicine-Bloomington, Bloomington, IN 47405, Bloomington, IN, Biochemistry and Molecular Biology, Indiana University School of Medicine-Bloomington, Bloomington, IN 47405, Bloomington, IN, Bloomington, IN*

[4] *Medical Sciences, Indiana University School of Medicine-Bloomington, Bloomington, IN 47405, Bloomington, IN*

Email: *davadelf@iu.edu*

Breast cancer is the most frequently diagnosed human cancer and is the second-leading cause of cancer deaths in women.Heat Shock Factor 1 (HSF1) is a transcription factor that plays a critical role in the heat shock response and the unfolded protein response. HSF1 activity has been shown to cause worseprognosis in breast cancer. Estrogen Related Receptor α (ERRα) is an orphan nuclear receptor that mitigates metabolic stress by altering mitochondrial biogenesis. It is unknown if these two pathways have any interaction. We have observed a cooperation and a physical interaction between HSF1 and ERRα. Co-expression of both HSF1 and ERRα led to changes in transcriptional activity of both transcription factors as assessed by luciferase reporter assays. Additionally, we observed a physical interaction between HSF1 and ERRα via co-immunoprecipitation.Leucine-rich nuclear binding domains have previously been seen to interact with nuclear receptors and several proteins interact with ERRα through this motif. ERRα interacts with its coactivator PGC-1αthrough a leucine-rich nuclear receptor binding motif (LXXLL, LXXXL, LXXL). We identifiedseveral novel nuclear receptor binding motifs in the HSF1 protein sequence. To determinethe function of this motif in HSF1, residues in these motifs were mutated by replacing the flanking leucineswith alaninesto renderthem non-functional. Sequencing data confirmed that mutagenesis of each of these nuclear receptor binding motifs was successful.These mutants can be used in subsequentstudies to discover a deeper understanding of the interaction of HSF1 with nuclear receptors. We will express ERRα and HSF1 wild-type or mutants in HEK-293 cells that have endogenous HSF1 knocked out and perform a co-immunoprecipitation to observewhich mutants affect HSF1-ERRα interaction.We will also co-express ERRα with wild-type or mutant versions of HSF1 and assess transcriptional activity of both HSF1 and ERRα to identifywhich HSF1 mutantshave lost their effect on ERRα. These studies identifya novel interaction between two significant transcription factors in breast cancer and future studies will elucidate the molecular underpinnings of their interaction.

Basic Science ***Undergraduate Student***

POSTER #63

COMPARING APERIO AND QUPATH USING THE ANALYSIS OF P53 BY IMMUNOHISTOCHEMISTRY IN SARCOMA PATIENTS

Danijela Bastaic[1,2]

[1] *Department of Pathology and Laboratory Medicine, Indianapolis, IN*
[2] *IU School of Medicine*

Email: *dbastaic@iu.edu*

Soft tissue sarcomas represent a diverse group of cancers originating in the body's soft tissues. These malignancies disproportionately affect children, comprising 15% of all cancers in individuals under 20 years of age, with an estimated 1,500 to 1,700 pediatric cases diagnosed annually in the United States. Encouraging advancements in treatment have resulted in five-year survival rates of approximately 65% for soft tissue sarcomas and 70% for bone cancers.

This study analyzed 50 clinical sarcoma samples sourced from the Indiana University Medical Center. Among these, 28 were primary tumors, and 22 were metastatic tumors. The analyzed subtypes included Liposarcoma, Leiomyosarcoma, Fibrosarcoma, Carcinosarcoma, Angiosarcoma, Rhabdomyosarcoma, Malignant Fibrous Histiocytoma (MFH), Malignant Peripheral Nerve Sheath Tumor (MPNST), Synovial Sarcoma, Osteosarcoma, and Ewing's Sarcoma. Tissue blocks were obtained from the Indiana University Health Pathology Laboratory under IRB-approved protocols and HIPAA-compliant informed consent. Most patients included in the study had received Adriamycin and Etoposide treatments prior to this research.

Immunostaining for P53, a tumor suppressor gene, was performed using the DAKO platform with the LSAB2 system at the IU Health Pathology Laboratory. The stained tissue slides were scanned digitally using the Aperio Imaging System, and the positive-pixel algorithm was used for quantitative analysis. Additionally, QuPath, an open-source software for quantitative pathology, was utilized for comparison to evaluate its flexibility in segmentation and tumor microenvironment visualization.

The Aperio analysis indicated that primary tumors exhibited a P53 positivity rate of 4.83% in tumor cell nuclei, compared to 4.65% for metastatic tumors. In contrast, QuPath revealed higher positivity rates, with 16.70% for primary tumors and 15.64% for metastatic tumors. QuPath’s advanced segmentation and visualization capabilities provided deeper insights into tumor microenvironments, suggesting its potential as a complementary tool to Aperio’s automated analysis.

In conclusion, while differences were noted in P53 positivity rates between primary and metastatic sarcomas, the results highlight the value of leveraging both Aperio and QuPath for enhanced analysis. These complementary methodologies offer promise for more precise and comprehensive studies of sarcoma biomarkers in the future.

Basic Science ***Undergraduate Student***

POSTER #64

EXPLORING THE IMPACT OF DUAL TARGETING APE1'S MAJOR FUNCTIONS AND THE IMPLICATIONS IN PDAC TREATMENT

Katherine Brady[1,2]

[1] *IUI School of Science, Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN*
[2] *IUSM*

Email: *kebrady@iu.edu*

Pancreatic ductal adenocarcinoma (PDAC) is a notoriously lethal cancer diagnosis marked by a poor 5-year survival rate and a strong resistance to conventional therapies. Its resilience is largely due to an ability to adapt to hypoxia, oxidative stress, and DNA-damaging conditions, hallmarks of its harsh tumor microenvironment. A key mediator of these adaptive responses is the multifunctional protein apurinic/apyrimidinic endonuclease 1/redox effector factor 1 (APE1/Ref-1). APE1 plays a critical dual role in cancer biology, functioning in the base excision repair (BER) pathway to maintain genome stability, and in redox regulation to control transcription factors that drive tumor and survival pathways. APE1 is frequently overexpressed in aggressive cancers such as PDAC, where it enhances tumor progression and therapeutic resistance. Targeting its redox function with small molecule inhibitors has shown promise in preclinical models, as has disruption of its endonuclease activity. However, while the individual roles of each domain have been studied extensively, the consequences of dual functional loss remain poorly characterized largely due to its critical role in cell survival which has made it difficult to target it. To address this gap, we have generated a novel APE1 double mutant with specific point mutations that separately target its DNA repair and redox regulatory functions. This study focuses on the phenotypic and functional characterization of this double mutant in a PDAC cellular model. We aim to evaluate how simultaneous disruption of both domains affects cell viability, DNA repair capacity, oxidative stress response, transcriptional regulation, and sensitivity to chemotherapeutic agents aimed at its redox and BEr functions. Through examining the combinatorial impact of this dual-function loss, this work builds upon our prior single-mutant studies and provides a more comprehensive understanding of APE1's role in maintaining PDAC homeostasis. These findings will offer new insights into the feasibility and therapeutic relevance of targeting both functions of APE1 simultaneously, potentially paving the way for more effective strategies to overcome resistance in pancreatic cancer.

Basic Science ***Undergraduate Student***

POSTER #65

PP2A ACTIVATION IN PDAC: ROLES OF AREG AND STRESS RESPONSE

Roe Chianis[1]

[1] *Purdue University Biological Sciences*

Email: *echianis@purdue.edu*

Pancreatic ductal adenocarcinoma (PDAC) has the worst five-year survival rate of all major cancers at only 13%. Activating mutations in the small GTPase, KRAS, are the driving mutations in PDAC, butit is known that upstream wild type EpidermalGrowthFactorReceptor (EGFR) signaling is requiredfor PDAC initiation and progression. Currently, EGFR inhibitors are FDA approved for PDAC and KRAS inhibitors are in clinical trials. However, these compounds against EGFR and KRAS have shown limited improvement on patient survival or high rates of resistance. Protein phosphatase 2A (PP2A) negatively regulates many of the downstream effectors in the EGFR-KRAS pathwaybut has decreased activity in PDAC patients, making reactivation a promising alternative therapeutic strategy. However, unpublished data from our lab has identified a feedback loop where PP2A activation leads to an increase in tumor promotionalphenotypes in an amphiregulin (AREG) dependent manner. AREG is an EGFR ligand that is also heavily implicated in therapeutic resistance through many mechanisms including modulation of the cellular stress response. Cellular stress response is altered with PP2A activation. This study aims to determine the roles of AREG and stress response genes in cell death induced by PP2A activation.

Basic Science ***Undergraduate Student***

POSTER #66

MODULATING AN ADAPTIVE STRESS RESPONSE DUALLY TARGETS HEMATOPOIETIC STEM CELLS AND ACUTE MYELOID LEUKEMIA TO IMPROVE TRANSPLANTATION OUTCOMES

Tania D. Lloyds[1,3], Abdullahi Mobolaji Idowu[2], Stephanie N. Hurwitz[2]

[1] *Melvin and Bren Simon Comprehensive Cancer Center, Indiana University, Indianapolis, IN*
[2] *Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Melvin and Bren Simon Comprehensive Cancer Center, Indiana University, Indianapolis, IN*
[3] *Department of Pathology and Laboratory Medicine, Indiana University School of Medicine*

Email: *tlloyds@iu.edu*

Hematopoietic stem and progenitor cells (HSPCs) are essential for lifelong blood cell production, and their transplantation serves as a therapeutic option for patients with blood disorders, including acute myeloid leukemia (AML). However, during chemotherapy and post-transplantation inflammation, HSPCs can become exhausted, leading to poor long-term blood production and increased complications. Recent work from our lab has shown that treatment with a small molecule GW4869 (GW) activates the integrated stress response (ISR), which improves long-term HSPC function by training cell tolerance to inflammation; however, the mechanisms underlying this cellular memory are unknown. In addition, the impact of this stress response on malignant HSPC (leukemia cell) resilience is not well understood.

Here we utilize short-term ex vivo expansion cultures of both murine and human HSPCs to demonstrate that GW induces epigenetic changes in cells that likely result in transcriptional repression of inflammatory genes. Striking variations in chromatin condensation measured by a DNase hypersensitivity assay were observed in HSPCs at various timepoints after treatment, suggesting that GW induces significant changes in temporal chromatin structure. Chromatin remodeling and adaptive transcriptional reprogramming of cells is likely in part driven by changes in histone H3 post-translational modifications (HPTMs), including H3 methylation and acetylation that were measured in HSPCs after GW treatment.

Parallel work in our lab has investigated ISR activation in AML chemoresistance. Cancer cells, including AML experience substantial cellular stress and frequently rely on the ISR for cell survival. Here we demonstrate dose-dependent cytarabine (AraC)-mediated activation of the ISR in chemoresistant AML cell lines MOLM-14 and HL-60. Pretreatment of AML cells with a pharmacologic ISR inhibitor (ISRIB) sensitized cells to AraC, resulting in reduced cell viability.

Altogether, these findings identify a conserved adaptive stress response in normal HSPCs and AML cells that impacts cell fitness. Our ongoing work aims to further investigate cellular mechanisms of ISR training, with the goal of strategically modulating this axis to achieve dual therapeutic benefits: sensitizing AML cells to chemotherapy and enhancing long-term preservation of HSPC function after transplantation.

Basic Science ***Undergraduate Student***

POSTER #67

IMPACT OF NXP800 DRUG TREATMENT ON CANCER CELL PHENOTYPES VIA THE HSF1 AND GCN2 PATHWAYS

Julie Heldman[2], Haddie DeHart[1], Natasha Hockaden[1], Richard Carpenter[1]

[1] *Indiana University, Bloomington, IN*
[2] *Indiana University*

Email: *juheldma@iu.edu*

Breast cancer is the most prevalent form of cancer in humans. In the early stages of the disease, the survival rate is near 99%. However, as the cancer metastasizes during more advanced stages, this survival rate drops to just 30%. HSF1 is a transcription factor responsible for implementing the heat shock response in cells under environmental stress. It has become clear over the last several decades that HSF1 is essential for tumor formation due to its roles in proteostasis. Furthermore, HSF1 has also been found to have several cancer-specific functions such as epithelial-to-mesenchymal transition (EMT), enhancing DNA repair, and suppressing immune-mediated killing, among many others. Therapies targeting HSF1 have not been successful enough to reach clinical trials. However, a novel inhibitor, called NXP800, was initially identified by screening for compounds that would suppress HSF1 activity. However, treatment with NXP800 is also known to increase activity of the GCN2 pathway, which is part of the integrated stress response pathway. Our results indicate NXP800 treatment leads to a potent decrease in HSF1 protein. The specific interaction between the GCN2 and HSF1 pathways is currently unknown, as is the impact of the drug on phenotypes of breast cancer cell lines. Results indicate NXP800 potently suppresses cancer cell migration and colony formation. We further observed that inhibition of GCN2 with addition of NXP800 led to a partial rescue of HSF1 protein, indicating a mechanistic link between GCN2 signaling and HSF1. Further studies with NXP800 will both help clarify the molecular connection between GCN2 and HSF1, as well as identify its therapeutic promise for breast cancer.

Basic Science ***Undergraduate Student***

POSTER #69

COMPARING THE ROLE OF PP2A-B56A AND PP2A-B55A ACTIVATION IN EGFR SIGNALING IN PDAC

Emma Kay, Claire Pfeffer, Brittany Heil, Roe Chianis, Sydney Clifford, Brittany Allen-Petersen

Email: *kay17@purdue.edu*

Pancreatic Ductal Adenocarcinoma (PDAC) has the worst 5-year survival rate of all major cancers, therefore there is an urgent need for further investigation and research. 90% of all PDAC tumors are driven by a mutation in KRAS, but wildtype EGFR activation is required for tumor oncogenesis. Protein Phosphatase 2A (PP2A) is a serine/threonine phosphatase that can negatively regulate downstream effectors of KRAS. Because of this, PP2A is highly important when considering potential PDAC therapeutic strategies. The PP2A complex is made up of 3 subunits: scaffolding (A), regulatory (B), and catalytic (C). There are many different regulatory B subunits, and the function of the PP2A complex is determined by which particular B subunit is incorporated. In the field, there is a lack of study of the regulatory B unitsand their effects of PP2A activation.Our lab has found that B56a, previously described as a tumor suppressor, actually promotes tumorigenic phenotypes through the EGFR signaling pathway when it is overexpressed.Todetermine if this PP2A-driven activation of EGFRsignaling and tumorigenic phenotypes is specific to PP2A-B56a, we made a cell line that overexpresses B55a, which is from a different family but has been implicated as a subunit with both tumor suppressive and tumor promotional phenotypes depending on the tissue.Phenotypes of this cell line, including proliferation, clonogenic colony formation, and anchorage independent growth, were compared to B56a. We found that PP2A-B55a does not replicate the increase in tumorigenic phenotypes of the PP2A-B56a complex, suggesting that these changes are specific to PP2A-B56a.

Basic Science ***Undergraduate Student***

POSTER #70

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

Kaitlyn Mills[1]

[1] *Indiana University Bloomington*

Email: *kaimills@iu.edu*

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 ETV1 and EWS, an RNA binding protein and transcriptional coactivator that promotes ETS activity.

Our lab has established that ETV1, ETV4 and ETV5 form essential interactions with EWS. More specifically, ETV4 and ETV5 form a direct interaction with EWS, and ETV1 forms an indirect interaction with EWS. It is also known that chromosomal rearrangements frequently result in N- terminal truncations of ETV1 in prostate cancer tumors. Co-immunoprecipitation assays utilizing whole cell lysate containing tETV1 and purified EWS has shown retention of the EWS interaction domain. Further, tETV1 remains oncogenic, as it promotes both cellular migration and colony formation in vitro. 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 #71

FORMULATION DEVELOPMENT FOR APURINIC/APYRIMIDINIC ENDONUCLEASE 1/REDOX EFFECTOR FACTOR 1 (APE1/REF-1) INHIBITORS FOR ENHANCED ORAL BIOAVAILABILITY

Mansi Nayak[1]

[1] *School of Science, Indiana University Indianapolis*

Email: *nayakm@iu.edu*

APE1/Ref-1 is a multifunctional protein that plays a dual role in DNA base excision repair and redox regulation of transcription factors. While critical for normal cellular function, APE1/Ref-1 is frequently overexpressed in aggressive cancers such as pancreatic, breast, and prostate cancers, correlating with poor prognosis. Pancreatic ductal adenocarcinoma (PDAC) remains especially challenging, with a five-year survival rate of approximately 12.5%, largely due to late-stage diagnosis and aggressive tumor biology. Although surgical resection offers the best chance for long-term survival, only 20% of patients are eligible at diagnosis.

Approximately 40% of new chemical entities and 65% of oral anticancer drugs are poorly water-soluble, leading to reduced bioavailability and therapeutic efficacy. Our laboratory has developed several naphthoquinone-based APE1/Ref-1 inhibitors and demonstrated their efficacy in both in vitro and in vivo PDAC models. However, the poor aqueous solubility of these compounds limits their gastrointestinal absorption, necessitating higher doses which could result in adverse reactions.

To address this, we have formulated these compounds using FDA-approved excipients and principles derived from amorphous solid dispersions and self-emulsifying drug delivery systems—approaches successfully applied to over 50 marketed drugs. Our formulations have shown enhanced solubility and were well tolerated in experimental animals. Currently, we are conducting short-term stability studies in 0.1N NaOH, HCl, PBS, and water, and are preparing to evaluate the pharmacokinetics and therapeutic performance of these novel formulations in mice under IACUC-approved protocols.

Basic Science ***Undergraduate Student***

POSTER #72

OPTIMIZATION OF THE MURINE HINDLIMB LYMPHEDEMA MODEL

Christopher Subi-Kasozi[1,2]

[1] *Department of Radiation Oncology, Radiation and Cancer Biology Laboratories, and Department of Medical & Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, Indianapolis, IN*
[2] *Division of Plastic Surgery, Indiana University School of Medicine*

Email: *csubikas@iu.edu*

Introduction:

Post-surgical lymphedema frequently occurs following lymph node dissection. The murine tail is the most commonly used model to study secondary lymphedema. The murine hindlimb model offers a more clinically translatable approach but results in the literature have been inconsistent. The purpose of this study is to 1) optimize the murine hindlimb lymphedema model to achieve consistent results and 2) assess the utility of radiation on the model.

Methods:

C57BL/6 mice underwent either 20-Gy irradiation of one hindlimb 7 days prior to surgery (n=11) or no radiation (n=9). For all mice, a circumferential skin incision was created at the proximal hindlimb. Lymphatics were identified with isosulfan blue dye and disrupted. Popliteal lymph nodes were excised. Paw thickness was measured and near-infrared laser lymphangiography was used to assess lymphatic function.

Results:

The average paw thickness of the operated hindlimb in irradiated mice on postoperative day (POD) 14 was 3.5±0.3 cm compared to 2.1±0.05 cm on the contralateral limb (p=0.0001). Lymphangiography on POD-42 showed significantly worse lymphatic function in the operated hindlimb compared to the control hindlimb (p=0.003). For the non-radiated mice, the paw thickness was 2.5±0.2 cm on POD-42 compared to the contralateral limb (2.1±0.1 cm) (p=0.0002) but smaller than radiated hindlimb group (3.2±0.1 cm) (p=0.0002). The nonradiated mice had significantly greater paw thickness than the control limb until POD-56 whereas the radiated mice sustained significant paw thickness to POD-90.

Conclusion:

Radiation of the murine hindlimb model results in sustained lymphedema compared to non-irradiated mice. The murine hindlimb lymphedema model is clinically more translatable than the murine tail model with consistent results.

Basic Science ***Undergraduate Student***

POSTER #73

EXPERIMENTAL EVALUATION OF DERMAL LYMPHATICS IN LYMPHEDEMA PREVENTION

Steven Sullivan[2], Luci Hulsman BS[1], Ganesh Mohan PhD[1], Shahnur Ahmed MD[1], Ayah R. Mahariq[1], Miguel Jorge BA[1], Mithun Sinha PhD[1], Aladdin H. Hassanein MD, MMSc[1]

[1] *Division of Plastic Surgery, Indiana University School of Medicine, Indianapolis, IN, Indianapolis, IN*
[2] *Division of Plastic Surgery, Indiana University School of Medicine, Indianapolis, IN*

Email: *stejsull@iu.edu*

BACKGROUND:

Secondary lymphedema is characterized by limb swelling following lymphatic disruption. Lymphedema results in slowed transition of lymph through the lymphatic collecting ducts and dermal backflow in the subdermal lymphatics. The role of dermal lymphatics in the development of lymphedema is poorly understood. The purpose of this study is to evaluate the effect of dermal lymphatic preservation in the development of lymphedema in a murine tail experimental model.

METHODS:

A standard murine lymphedema tail model was used as the study control. This involved a 3 mm circumferential excision 20 mm from the base of the tail. Both lymphatic channels adjacent to the veins were clipped (Control n=6). The experimental group was a modification of the standard model consisting of a 3 mm hemi circumferential excision and a mirrored hemi circumferential excision, 3 mm proximal to the first site (Experimental n=8). This experimental surgical design resulted in the disruption of both large lymphatic vessels with preservation of a dermal bridge between the two surgical sites. Serial tail volume was assessed at days 7, 14, 21 and 28 using caliper tail measurements calculated with the truncated cone equation. Near infrared indocyanine green (ICG) laser lymphangiography was performed for functional assessment of lymphatic clearance.

RESULTS:

The experimental group had significantly lower change of tail volume at all measurement time points of day 7 (66.2 mm^3, 24.2 mm^3), day 14 (112.0 mm^3, 11.5 mm^3), day 21 (149.6 mm^3, 5.1 mm^3), and day 28 (158.1 mm^3, 15.6 mm^3) all $p<0.001$. Near infrared laser lymphangiography demonstrated improved lymphatic clearance in the experimental group marked by decreased ICG dye intensity at hours 6, 24, 48, 72, and 96 ($p<0.001$).

CONCLUSION:

This study shows dermal lymphatics can preserve of lymphatic function in this experimental model. Current clinical surgical prevention measures include immediate lymphatic reconstruction with lymphovenous anastomosis after axillary dissection and vascularized lymph node transfer. Dermal lymphatics may also have the potential to be leveraged for lymphedema prevention at the lymphatic injury site.

Basic Science ***Undergraduate Student***

POSTER #75

SKIN CANCER RISK BEHAVIORS FROM THE 2022 HEALTH INFORMATION NATIONAL TRENDS SURVEY (HINTS 6)

Eric Walsh-Buhi[1,3], Hannah Javidi[2], Alexandra Hughes-Wegner[2]

[1] *Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indiana University Center for Community Engaged Dissemination and Implementation Research, Bloomington, IN*
[2] *Indiana University School of Public Health-Bloomington, Bloomington, IN*
[3] *Indiana University School of Public Health-Bloomington*

Email: *erwals@iu.edu*

Introduction. Skin cancer prevalence is increasing globally, with UV exposure being a primary risk factor. Skin cancer incidence and mortality can be reduced through improved prevention and communication efforts; therefore, it is crucial to understand factors that influence engagement in risky sun behaviors. This study assesses skin cancer risk behaviors and their predictors.

Methods. A nationally representative U.S. sample (N=5,604) from the 2022 Health Information National Trends Survey (HINTS 6) was analyzed to examine past-year sunburns and alcohol consumption at any sunburn. Multivariable analyses included sociodemographic predictors (sex at birth, age, race/ethnicity, sexual orientation, education, income), with pairwise comparisons to explore subgroup differences.

Results. Approximately one-third of the sample (N=1,817, 32.6%) reported at least one sunburn in the past year and, of those individuals, 21.8% (N=396) reported alcohol consumption at any sunburn. Sunburn rates were highest among 18-34-year-olds (53.1%), Non-Hispanic Whites (42.2%), and gay/lesbian (47.4%) and bisexual (51.5%) individuals. Men (34.9%) had higher sunburn rates than women (30.6%), but White women aged 18-34 years reported the most sunburns in the past year (77.1%). High-income (≥$75,000; 43.8%) and college-educated individuals (40.0%) had highest sunburn rates. Sunburn during alcohol-use was highest among non-Hispanic whites (22.4%) and Hispanics (22.5%); similar rates were seen amongst gay/lesbian (30.1%) individuals. Sex at birth, sexual orientation, education, race/ethnicity, income, and age (all $p<.001$) remained statistically significant predictors of a past-year sunburn.

Discussion. Despite prevention efforts, sunburn remains prevalent, especially among Non-Hispanic Whites, LGBTQ+ individuals, and young women. Targeted interventions are needed to improve awareness and protective behaviors.

Behavioral ***Faculty***

POSTER #76

CORRELATES OF DECREASED FEAR OF CANCER RECURRENCE AMONG BREAST CANCER SURVIVORS PARTICIPATING IN A THREE-ARM RANDOMIZED CONTROLLED TRIAL

Betsey Zenk Nuseibeh[1,2], Matthew Hays[3], Yang Li[3], Shelley A. Johns[3,4]

[1] *Indiana University, School of Public Health, Bloomington, IN*
[2] *Predoctoral Fellow, Interdisciplinary Training In Cancer Prevention and Control (T32CA117865)*
[3] *Indiana University School of Medicine, Indianapolis, IN*
[4] *Center for Health Services Research, Regenstrief Institute, Inc., Indianapolis, IN*

Email: *bznuseib@iu.edu*

Background/Purpose

Forty percent of breast cancer survivors (BCS) report clinical fear of cancer recurrence (FCR), negatively impacting quality of life. While cognitive behavioral therapy (CBT) and acceptance and commitment therapy (ACT) may effectively decrease FCR, most trials are in person. Videoconference FCR interventions may be more accessible. We examined the relationship between session attendance, session engagement, and between-session skills practice as correlates of decreased FCR for BCS participating in a 3-arm randomized controlled trial comparing videoconference CBT, ACT, and survivorship coaching (SC).

Methods

BCS with clinical FCR (*N*=384, mean age=56.1 years [*SD*±11.3]) completed Fear of Cancer Recurrence Inventory-Short Form (FCRI-SF) at baseline and were randomly assigned to CBT (6 sessions), ACT (6 sessions), or SC (1 session). FCRI-SF was also measured at 2-, 6-, and 12-months post baseline (T2, T3, and T4). Univariable regression stratified by intervention was conducted at each time point compared to baseline using the independent variables session attendance, engagement, and skills practice.

ResultsFor CBT participants, engagement was significantly related to decreased FCRI-SF at T2 (T= -2.07, p=0.04); each unit increase in engagement (ranged 0-10) was related to decreased FCRI-SF by 0.9 units. For ACT participants, skills practice was significantly related to decreased FCRI-SF at T2 (T=-2.11, p=0.04) and T4 (T=-2.20, p=0.03); every 2-hours of additional skills practice per week was related to decreased FCRI-SF by 0.18 and 0.22 units at T2 and T4, respectively. For SC participants, neither engagement nor skills practice was significantly related to FCRI-SF. In all groups, no relationship was observed with attendance.

Conclusions

For FCR interventions delivered via videoconferencing, results suggest that decreased FCR may be related to active engagement in session activities in CBT and to between-session coping skills practice in ACT. Findings can inform development of intervention designs centered on the lived experiences of BCS.

Behavioral ***Graduate Student***

POSTER #77

A PATIENT-CENTERED DESIGN APPROACH TO IMPROVING MEDICATION SELF-MANAGEMENT FOR PATIENTS WITH BREAST CANCER USING ORAL ANTICANCER MEDICATIONS

Yejin Seo[6], Karen Suchanek Hudmon[1], Kellie Jones Weddle[2], Yuehwern Yih[3], Kathy Miller[4], Ephrem Abebe[5]

[1] *Purdue University, College of Pharmacy, Department of Pharmacy Practice, West Lafayette, IN*
[2] *Purdue University, College of Pharmacy, Department of Pharmacy Practice, Indianapolis, IN*
[3] *Purdue University, College of Engineering, Edwardson School of Industrial Engineering, West Lafayette, IN*
[4] *Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN*
[5] *Purdue University, College of Pharmacy, Department of Pharmacy Practice, Indiana University, School of Medicine, Indianapolis, IN*
[6] *Purdue University, College of Pharmacy, Department of Pharmacy Practice*

Email: *seo10@iu.edu*

Background

Developments in cancer therapeutics have led to increased use of oral anticancer medications (OAMs) for the treatment of breast cancer. The most common cancer among women in the U.S., patients with breast cancer often face challenges in managing OAM use at home. Designing interventions that enhance the patient experience of OAM use requires a deeper understanding of barriers faced by patients as they navigate their cancer care journey. The objective of this study was to (a) identify unmet medication management needs of patients with breast cancer receiving OAMs and (b) develop early prototypes to support medication management.

Methods

The study was conducted in two phases. For both phases, participants were recruited from a federally qualified health center's breast cancer clinic in central Indiana. Eligible patients were 18 years of age or older, diagnosed with breast cancer, and currently receiving OAMs. To characterize the patient experience with OAMs, Phase 1 involved conducting semi-structured interviews guided by journey mapping techniques . Visual storyboards were created to illustrate medication use experiences, highlighting key timelines, barriers, and facilitators. Personas were developed based on shared characteristics: 1) those on specialty medications (requiring specialty pharmacies); and 2) on traditional medications (available at community pharmacies). Phase 2 involved three rounds of participatory design workshops, focused on creating prototype tools addressing the identified challenges. In round one (inspiration stage), participants defined the problem space and prioritized challenges. In round two (ideation stage), they generated multiple solution ideas. In round three (convergence stage), two design concepts were developed and evaluated.

Results

In phase 1, 12 individuals (11 females, 1 male; median age 65.5 years, range 37–75) participated in an interviewed. Four had received specialty medications (palbociclib, ribociclib), and eight had received traditional medications (tamoxifen, anastrozole, exemestane). Specialty medication users reported challenges navigating insurance, while traditional medication users did not. Side effects were common across both groups, and two cases of suboptimal adherence were reported in the traditional group. In phase 2, the participatory design sessions were conducted with five participants forming the design panel. The median age was 66 years (range: 38–75). Participants identified key challenges, including difficulties navigating resources and information and managing medication side effects. The design panel prioritized two design concepts, which were subsequently developed into two prototypes: (1) a physical breast cancer handbook, and (2) an interactive treatment navigation app for use on tablet and smartphone devices.

Conclusion

This study provides insights into the patient experience with OAMs. The personas created can be applied in designing interventions tailored to breast cancer patients' needs and goals, while the consolidated journey maps identify potential areas for improvement. Future studies are needed to further develop and validate these prototypes.

Behavioral ***Graduate Student***

POSTER #78

EXPLORING MULTIMORBIDITY AND HEALTH OUTCOMES IN BREAST CANCER SURVIVORS: INTERSECTIONAL IMPACTS OF RACE AND OBESITY

Kelsey Sinclair[1], Misty Hawkins[2], Shelley Johns[3,4]

[1] *Appied Health Sciences, School of Public Health, Indiana University*
[2] *Health and Wellness Design, School of Public Health, Indiana University*
[3] *Indiana University School of Medicine*
[4] *Center for Health Resources Research, Regenstrief Institute*

Email: *kelsinc@iu.edu*

Background. Multimorbidity, defined as the co-occurrence of at least two chronic conditions, has significant consequences including poorer global functioning, lower health-related quality of life, increased mental health conditions, and a greater risk of premature mortality. Cancer survivors, particularly breast cancer survivors (BCS), are at an increased risk of experiencing multimorbidity due to previous diagnoses, treatment, and lifestyle changes. Obesity has been associated with an increased risk of developing multimorbidity and in BCS has been associated with greater risk of recurrence and premature mortality.

Methods. This study is a secondary analysis of the Facilitating Adaptive Coping with Fear of Recurrence Among BCS (FACing Fear) trial (NCT05364450) with a sample of 363 BCS (54 Black, 309 White). Data were collected at baseline, including demographics, multimorbidity, and health outcomes. Multimorbidity was aggregated into a total score comprised of 16 common comorbid conditions among cancer survivors. Participants were organized into four groups based on race and BMI status. Participants with a BMI of 30 kg/m^2 or higher were considered to have obesity. Outcomes included the PROMIS Global Health mental and physical global health subscales and total multimorbidity. Lower scores indicated worse global health and higher incidence of multimorbidity.

Results. One-way ANOVA analyses were conducted to determine differences between four groups of BCS: White with no obesity, Black with no obesity, White with obesity, and Black with obesity. Results revealed no significant differences between the four groups regarding multimorbidity incidence or global mental health. Significant group differences in global physical health were found among the White with no obesity group and the three other groups. Compared to White BCS without obesity ($X= 48.70$), Black BCS without obesity reported significantly worse global physical heath ($X= 43.95$, $p = .03$). Both groups with obesity also reported significantly worse global physical health compared to White BCS without obesity (White with obesity: $X= 43.54$, $p < .001$; Black with obesity: $X= 41.15$, $p <.001$). There were no significant differences between Black BCS with or without obesity and no significant differences between White and Black BCS with obesity.

Conclusions. This study highlights disparities related to race and obesity status. Regardless of race, obesity was related to significant negative impacts on physical health. However, race, regardless of weight status, was also significantly related to worse physical health. These findings highlight a need for targeted interventions and clinical care strategies to address these disparities and improve health outcomes for Black BCS and BCS living with obesity.

Behavioral ***Graduate Student***

POSTER #79

FINANCIAL TOXICITY AND PERCEIVED INJUSTICE: LONGITUDINAL ASSOCIATIONS WITH SYMPTOM CHANGES IN CANCER PATIENTS

Stella Snyder[3], Shelley Johns[1], Catherine Mosher[2]

[1] *Department of Medicine, Indiana University School of Medicine, Center for Health Services Research, Regenstrief Institute, Inc., Indianapolis, IN*
[2] *Department of Psychology, Indiana University Indianapolis, Indianapolis, IN*
[3] *Department of Psychology, Indiana University Indianapolis*

Email: *stelsnyd@iu.edu*

Greater perceived financial toxicity and injustice related to the cancer experience have been associated with higher levels of physical and psychological symptoms in cross-sectional studies. However, scarce longitudinal research has linked these perceptions to changes in physical and psychological symptoms to inform future interventions. This study begins to fill this gap by examining associations of financial toxicity and perceived injustice with changes in physical and psychological symptoms in solid tumor patients over a 2-month period.

Patients (N=177) with stage I-III breast, gastrointestinal, lung, or prostate cancer who reported at least mild financial toxicity were recruited from Indiana hospitals. Patients were either undergoing or had recently completed cancer treatment. Patients completed the Comprehensive Score for Financial Toxicity (COST), the Injustice Experience Questionnaire (IEQ), and PROMIS measures of pain, fatigue, sleep disturbance, anxiety, and depressive symptoms at baseline and 2 months later. The retention rate at follow-up was 94.3%. We used path analysis to examine relationships between baseline financial toxicity and perceived injustice and symptom changes, while adjusting for demographic and medical covariates.

Participants were primarily non-Hispanic White (78.5%) or Black (15.8%) and female (53.1%), with an average age of 62 years. Most participants (68.9%) had some college education, and 55% reported an annual household income of less than $51,000. Nearly half (46.9%) had stage III cancer, with diagnoses distributed fairly evenly across the cancer types. Higher financial toxicity was associated with worsening depressive symptoms (β=-0.19, p<0.01), but was not significantly correlated with changes in anxiety or physical symptoms. Greater perceived injustice was significantly associated with worsening anxiety (β=0.21, p<0.01), depressive symptoms (β=0.27, p<0.01), and sleep disturbance (β=0.13, p=0.02), and marginally associated with fatigue (β=0.09, p=0.08). Baseline symptoms were strong predictors of the same symptoms at follow-up (βs=0.44-0.65, ps<0.01).

Findings suggest that financial toxicity and perceived injustice are risk factors for depressive symptoms and that perceived injustice may also predict anxiety and physical symptom burden in patients with cancer. Further research is needed to explore the mechanisms underlying these associations to inform strategies to improve patients' financial well-being and symptoms.

Behavioral ***Graduate Student***

POSTER #80

EVALUATION OF INHIBITORS OF ENDOCANNABINOID DEACTIVATION ON THE DEVELOPMENT OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY AND TUMOR PROGRESSION IN A MOUSE BREAST CANCER MODEL

Jonah Wirt[1,5], Emily Fender-Sizemore[2], Mirjam Huizenga[3], Mario Van der Stelt[3], Andrea G. Hohmann[4]

[1] Program In Neuroscience, Indiana University, Bloomington, IN, Bloomington, IN
[2] Dept. of Psychological and Brain Sciences, Indiana University, Bloomington, IN, Program In Neuroscience, Indiana University, Bloomington, IN, Bloomington, IN
[3] Department of Molecular Physiology, Leiden University & Oncode Institute, Netherlands, Leiden, Netherlands,
[4] Dept. of Psychological and Brain Sciences, Indiana University, Bloomington, IN, Program In Neuroscience, Indiana University, Bloomington, IN, Gill Institute for Neuroscience, Indiana University, Bloomington, IN, Bloomington, IN
[5] Dept. of Psychological and Brain Sciences, Indiana University, Bloomington, IN

Email: *jlwirt@iu.edu*

Chemotherapy produces anti-cancer effects in breast cancer butalso produces dose-limiting side effects such as neuropathic pain. Inhibition of the primary enzymes that degrade endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA) suppress neuropathic pain behavior in models of chemotherapy-induced peripheral neuropathy (CIPN) while also displaying anti-cancer properties. 2-AG is hydrolyzed by the enzyme monoacylglycerol lipase (MGL), while AEA is degraded by fatty-acid amidehydrolase (FAAH), respectively. Our groups previously discovered anovel CNS impermeable MGL inhibitor, LEI-515, that was able to reverse neuropathic nociception in paclitaxel-treated mice in the absence of cancer. In the current studies, we assessed peripheral MGL inhibitor LEI-515, global MGL inhibitor JZL184, and peripheral FAAH inhibitor URB937 for their respective abilities to block development of CIPN and reduce tumor size in a mouse model of breast cancer. First, LEI-515, JZL184, URB937 or vehicle was administered prophylactically with the chemotherapeutic agent paclitaxel in a cohort of non-tumor bearing mice. Mechanical (assessed using the von Frey assay) and cold (assessed using the acetone test) hypersensitivities were then assessed. A separate cohort of BALBc mice received 4T1 tumor cell inoculation into the mammary fat pad.Pharmacological treatments began once tumors were palpable. Treatments were administered once daily for LEI-515, JZL184, URB937, or their vehicle, before paclitaxel treatment. Paclitaxel treatment was every two days. Mechanical and cold hypersensitivities were assessed every 4 days throughout the study, and tumor volumes were measured daily. At the terminal end point, tumor weight and colonic content in small and large intestine were assessed.Both peripheral MAGL inhibitor LEI-515 and peripheral FAAH inhibitor URB937 prevented the development of paclitaxel-induced mechanical and cold hypersensitivity in non-tumor mice. JZL184 did not prevent the development of mechanical or cold hypersensitivity induced by paclitaxel.LEI-515 did not interfere with the ability of paclitaxel to kill tumor cells in the MTT assay. In 4T1 tumor-bearing mouse studies, LEI-515 prevented the development of chemotherapy-induced mechanical and cold hypersensitivity, and reduced tumor size when combined with paclitaxel compared to chemotherapy alone. Global MGL inhibitor JZL184 reduced tumor size when combined with paclitaxel compared to chemotherapy alone, but did not prevent chemotherapy-induced mechanical or cold hypersensitivity. Peripheral FAAH inhibitor URB937 attenuated chemotherapy-induced mechanical and cold hypersensitivity butdid not enhance paclitaxel's ability to reduce tumor volumes when compared to paclitaxel alone. Paclitaxel mice that received LEI-515 showed reduced tumor weights. Colonic weights of all paclitaxel-treated mice increased.Our findings indicate that peripheral MGL inhibition holds therapeutic potential for cancer patients to treat both their cancer and CIPN. Our findings also indicate that peripheral FAAH inhibition holds therapeutic potential for the treatment of CIPN.

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Behavioral ***Graduate Student***

POSTER #81

THE PARENT EXPERIENCE OF PEDIATRIC STEM CELL TRANSPLANT

Elizabeth Harman[1]

[1] *School of Nursing*

Email: *ELHarman@iu.edu*

Introduction

It is well established that pediatric stem cell transplantation is a lengthy and difficult treatment, especially for infants and young children who make up approximately 50% of pediatric stem cell transplants in the United States. Supportive care interventions are needed to support these young children and their parents. However, there is limited foundational research about how parents (and young children) experience stem cell transplant. Before embarking on intervention development to support this vulnerable group of children and their parents, more information needed. This study is the first of two preliminary studies that lay the foundation for a larger intervention development study funded by the National Cancer Institute.

Objective

The objective of this study is to understand and describe the experiences of parents who have cared for their infant/young child through the HSCT experience.

Methods

We conducted semi-structured qualitative interviews (60-80 minutes in length) with parents of infants/young children (3 years of age and under) who have completed their HSCT in the last year.

Results

5 parents/primary caregivers of infants and toddlers who received an HSCT within the last year participated in interviews via phone. Themes related to caring for their child, caring for themselves, and managing the unknown emerged.

Conclusion

These data suggest that parents of young children undergoing HSCT face unique challenges and require creative strategies to support both the parent and their child. This data will be used to support the development of a music-based intervention for infants/young children undergoing HSCT and their parents.

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

POSTER #82

PREVALENCE OF CANNABIS AND MEDICATION USE BY INDICES OF RESIDENTIAL URBANICITY AND DEPRIVATION AMONG OHIO CANCER PATIENTS

Shieun Lee[1,3], Theodore Brasky[2], Alison Newton[2], Bella McBride[2], Ryan Baltic[2], Jessica Krok-Schoen[2]

[1] *Indiana University Simon Comprehensive Cancer Center, Bloomington, IN*
[2] *the Ohio State University College of Medicine , Columbus, OH*
[3] *Indiana University School of Nursing*

Email: *leeshie@iu.edu*

Background/Purpose: There is increasing interest in the use of cannabis products to alleviate symptom burden among cancer patients. Although data remain limited, there is some evidence to suggest that state legalization of cannabis is associated with reduced opioid use. Indices of area-level social determinants of health provide nuance to understanding the patterns of symptom-managing behaviors in the context of health equity.

Methods: Between July 2021 and July 2022, 943 adult cancer patients ages 18 or older, with an invasive cancer diagnosis at any anatomic site, who were treated at 8 clinics at the Ohio State University Comprehensive Cancer Center were recruited into an anonymous cross-sectional study. Eligible patients were identified in the electronic medical record prior to their clinic visit and recruited in clinic or by phone. For the present analysis, we restricted the sample to 854 patients who reported a valid Ohio residential zip code area to assign rural-urban commuting area (RUCA) codes and social deprivation index (SDI) values. RUCA was categorized as urban and micropolitan to rural and SDI was dichotomized at the median. Participants completed a one-time cannabis-focused questionnaire which included items on medications used to alleviate symptoms.

Results: The prevalence of self-reported cannabis (19.1% vs. 13.1%; P=0.02) and opioid use (29.9% vs. 20.5%; P<0.01) were higher among patients living in areas of higher vs. lower social disadvantage. When cannabis and opioids use were further examined, those living in a socioeconomically deprived area were more likely than their counterparts to use cannabis multiple times a day (12.4% vs. 5.7%; P<0.01) and medical cannabis without prescription (14.3% vs. 8.1%; P=0.02).

Conclusions/Implications: Larger, multi-institutional studies with detailed measurement of cannabis and medications, and an increased capacity to examine additional social determinants of health are needed to confirm and explain these descriptive findings.

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

POSTER #83

Remove

FEAR OF CANCER RECURRENCE AND COPING IN YOUNG BREAST CANCER SURVIVORS

Christa Torrisi[4], Matthew Hays[1], Yang Li[2], Shelley Johns[3]

[1] Department of Biostatistics and Health Data Science, Indiana University School of Medicine, Indianapolis, IN , Indianapolis, IN
[2] Indiana University School of Medicine, Indianapolis, IN , Indianapolis, IN
[3] Indiana University School of Medicine, Indianapolis, IN; Center for Health Services Research, Regenstrief Institute, Inc., Indianapolis, IN, Indianapolis, IN
[4] Indiana University School of Nursing, Indianapolis

Email: *ctorris@iu.edu*

Background & Purpose: Fear of cancer recurrence (FCR) is a disruptive problem for many breast cancer survivors (BCS). While prevalent across all ages, younger BCS (those diagnosed under the age of 45) often experience more intense FCR. This is characterized by excessive fear, worry, and functional impairment; consequently, FCR can adversely affect quality of life in younger BCS. Coping strategies can have a positive or negative impact on FCR. Adaptive coping using problem- and emotion-focused coping strategies alleviate FCR and enhance well-being across diverse survivor populations, while maladaptive avoidant coping can increase distress and FCR. Currently, specific coping mechanisms employed by young BCS, and how these may differ by race, remain understudied. We aimed to investigate the relationship between FCR and coping among young BCS.

Methods: Between 2021 and 2023, 88 early-stage (I-IIIA), post-treatment, young BCS were recruited from the Midwestern United States to complete a one-time survey. Validated measures were used to assess FCR (FCR-7), coping strategies (B-COPE), psychological flexibility (CAAQ), and mental health (PROMIS Global Mental). Mixed models regression was employed to examine the associations between coping and FCR.

Results: Mean age at breast cancer diagnosis was 41.7 years (SD=3.8). Most participants were white (81.8%), partnered (71.6%), and college educated (70.5%). Over 63% of participants had completed breast cancer treatment within the previous two years. Findings revealed that as FCR increased, young BCS reported less psychological flexibility (p=<0.001) and were more likely to employ avoidant coping strategies (p=0.0007) or problem-focused coping strategies (p=0.0068). Black young BCS were, on average, more likely to use avoidant coping compared to White young BCS (p=0.0055). Emotion-focused coping was used more, on average, in Black young BCS than White (p=0.0003) or other race/multiracial young BSC (p=0.0051). As mental health improved, young BCS were less likely to use maladaptive avoidant coping (p=<0.0001) and were more likely to display greater psychological flexibility (p=<0.0001).

Conclusions: FCR was associated with both adaptive and maladaptive coping in young BCS, with improved mental health associated with reduced maladaptive coping. Future research should explore the mechanisms of these associations and develop culturally-sensitive interventions to support alternatives to avoidant coping in young BCS and work to promote and support quality of life.

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

POSTER #84

Remove

TOWARDS UNDERSTANDING TRENDS PREVENTING PATIENT SUCCESS OUTCOMES IN ONCOLOGY CARE

Renee Kessler[2], Ayla Dimon[2], Jessica Kiebler[3], Ann C. Kimble-Hill[1,4]

[1] Office of Inclusive Excellence, Indiana University Simon Comprehensive Cancer Center, Indianapolis, IN, US , Indianapolis , IN
[2] Office of Inclusive Excellence, Indiana University Simon Comprehensive Cancer Center, Indianapolis, IN, US
[3] Department of Psychology, School of Science, Indiana University Indianapolis, Indianapolis, IN, US
[4] Department of Biochemistry & Molecular Biology, Indiana University School of Medicine, Indianapolis, IN, US

Email: *renkess@iu.edu*

Individuals who areimpacted by social determinants of health (SDH) account for 50% of cancer cases mortality. Research indicates that this and other cancer health disparities persist partly because physicians reinforce cultural biases and are not given adequate training to address SDH in oncology. This results in lower-quality care and worse health outcomes for people of color and those from economically disadvantaged areas, creating a critical need for physicians to gain the knowledge and skills necessary to overcome barriers in underserved communities, improve communication, and build trust with the patient population. The current study aims to understand whether having a more culturally competent clinical team will lead to improved patient experiences and outcomes. The sub-aims will examine 1) members involved on the treatment team; 2) important knowledge for healthcare providers; and 3) what additional resources would have improved patient care by examining survey data from patients, patient support, and clinicians. Study participants (N = 58) were recruited from social media and local flyer distribution and asked to complete a population-specific survey on Qualtrics. All three surveys consisted of standard demographic questions, followed by questions taken from the United Kingdom Cancer Awareness Measure (CAM) to examine help-seeking barriers from the perspective of each group. The last section of the survey measured familiarity and the impact of education, communication approaches, and SDH for diverse patients. Specific overlapping questions across the three surveys were used for comparative analysis to determine the level of agreement between populations. Participants identified seven themes related to patient-provider dynamics, six themes associated with training and education, and four themes related to the availability of resources.The current study illuminates shortcomings in medical care that reflect the literature on inconsistent training that results in healthcare inequities while highlighting new areas for intervention. Future research includes studying population-specific barriers and developing resources toimprove the quality of oncology care,patient experience, and health inequities.

Behavioral ***Postbaccalaureate Fellow***

POSTER #85

ACCEPTABILITY AND USABILITY OF A CO-DESIGNED STRESS MANAGEMENT APP FOR BREAST CANCER SURVIVORS: RESULTS FROM THE MOSAIC PILOT STUDY

Tayler Gowan[5], Evan Jordan[1], Betsey Zenk Nuseibeh[2], Madison Schwarz[3], Shelley Johns[4]

[1] *School of Public Health, Indiana University, Center for Health Services Research, Regenstrief Institute, Bloomington, IN*
[2] *School of Public Health, Indiana University, Bloomington, IN*
[3] *Indiana University School of Medicine, Indianapolis, IN*
[4] *Indiana University School of Medicine, Center for Health Services Research, Regenstrief Institute, Indianapolis, IN*
[5] *Indiana University School of Medicine*

Email: *taylergowan@gmail.com*

Background: Breast cancer is the most prevalent cancer globally, affecting over 7.8 million women. Despite a 91% five-year survival rate in the United States, more than half of breast cancer survivors experience elevated stress, anxiety, and depression post-treatment. Many lack access to mental health services due to financial constraints, provider shortages, and scheduling conflicts. Mobile health applications show promise for improving access to care. Acceptance and Commitment Therapy (ACT), an evidence-based behavioral approach, has been used effectively in mobile health applications and has been shown to improve the mental health of breast cancer survivors.

Objective: To improve care access for breast cancer survivors, this 3-phase study employed user-centered co-design methods to develop and test an ACT mobile health application targeting psychological distress.

Methods: Phase 1 involved 5 co-design sessions over about 2 months with 5 breast cancer survivors familiar with ACT, 3 ACT practitioners, and 2 user-centered design experts. Phase 2 focused on testing acceptability and usability. Feedback was collected through semi-structured focus group interviews, which were audio-recorded, transcribed, and qualitatively analyzed. Iterative improvements were made across 3 testing rounds with cohorts of 5 post-treatment breast cancer survivors (n=15; mean age 53.1 years [SD = 10.8]). Participants used the app for 3 weeks alongside a heart-rate variability (HRV) monitor that prompted app use when elevated stress was detected.

Results: Phase 1 themes included key stressors (e.g., fear of recurrence, relational and financial impacts), desired app content, and design preferences. In Phase 2, the app surpassed established benchmarks for acceptability and useability: the mean score on the Acceptability E-Scale was 26.1 (cutoff = 24), and the System Usability Scale score was 75.0 (cutoff = 68). Qualitative suggestions for app improvement fell into four main categories: 1) technical and functional challenges (e.g., app glitches, broken features), 2) navigation and organizational improvements (e.g., categorizing activities, streamlining layout), 3) design and content personalization (e.g., improving visual consistency, tailoring content), and 4) device and alert limitations (e.g., improving HRV alert accuracy, reducing phone interruptions). Survivor-informed improvements are underway so that the refined app version can be further pilot tested.

Conclusion: A user-centered process led to the development of an ACT-based mobile health application co-designed with breast cancer survivors. Prototype testing showed strong acceptability and usability, with refinements underway to enhance the user experience. Phase 3 will evaluate feasibility of a larger scale study of the app and assess effects on key outcomes (e.g., symptoms of depression, anxiety, stress).

Behavioral ***Research Coordinator***

POSTER #86

FEAR OF RECURRENCE IMPACT AND COPING AMONG TESTICULAR CANCER SURVIVORS: A QUALITATIVE EXPLORATION

Madison Schwarz[1], Michelle Hoy[1], Janet Panoch[1], Christa Torrisi[2], Jennifer R. West[3], Jay Baute[1], Deborah Buckles[4], Shelley A. Johns[1,3]

[1] *Indiana University School of Medicine, Indianapolis, IN*
[2] *Indiana University School of Nursing, Indianapolis, IN*
[3] *Center for Health Services Research, Regenstrief Institute, Indianapolis, IN*
[4] *Indiana University Simon Comprehensive Cancer Center, Indianapolis, IN*

Email: *madischw@iu.edu*

Background: Testicular cancer is the most common solid malignancy in men aged 14 to 40. Given the 95% 5-year survival rate for testicular cancer, survivors often face long-standing physical and psychological adverse health outcomes, including fear of cancer recurrence (FCR). Our team's survey of 117 testicular cancer survivors (TCS) found that 50% reported moderate to severe FCR using the validated FCR-7 measure. A lack of qualitative research exploring FCR among TCS has profoundly limited the development of FCR interventions for TCS. As part of a mixed methods pilot study, we conducted qualitative focus group (FG) interviews with TCS to explore their experiences with FCR, including its impact on TCS' lives and how they are coping.

Methods: Eligible participants were at least 18 years old, had completed testicular cancer treatment >2 weeks but ≤ 5 years before enrollment, and showed no evidence of active disease. A subset of 26 TCS who completed the quantitative survey participated in a semi-structured FG interview. Each FG (n=4) included approximately 7 participants and featured open-ended questions about FCR experiences in four domains: (1) triggers; (2) effects and manifestations; (3) wants and needs; and (4) coping mechanisms. Responses were recorded, transcribed, and thematically analyzed by 7 researchers to explore FCR among TCS.

Results: Among the 26 FG participants, the mean age was 30.8 (*SD*=5.3), and 61.5% completed their last round of TC treatment 2 to 5 years ago. Most FG participants (65.4%) reported moderate to severe FCR. Participants frequently described physical symptoms, memories of treatment, and upcoming medical appointments as triggers for FCR. They reported that FCR affected various aspects of life, including mental health, daily functioning, socioeconomic status, and future planning. Participants discussed using both maladaptive (e.g., avoidance, distraction, excessive online research) and adaptive (e.g., acceptance, self-reassurance, reliance on support systems) strategies to cope with FCR.Qualitative responses also included preferences for TC-specific interventions targeting FCR, such as increased patient education and peer support within the TCS community.

Conclusion: Findings suggest that many TCS with clinical FCR experience significant impacts on well-being without adequate coping strategies. Themes from this analysis have informed the development of an FCR intervention in collaboration with TCS, which will be tested in a pilot randomized controlled trial.

Behavioral ***Research Technician***

POSTER #87

PURSUING EXPLORATION INTO THE SUPPORTIVE CARE NEEDS AND INTERVENTION PREFERENCES OF SURVIVORS OF TESTICULAR CANCER (PERSIST): PHASE 1 RESULTS FROM A 3-PHASE PILOT STUDY

Jennifer West[3], Matthew Hays[1], Yang Li[1], Shelley Johns[2]

[1] *Indiana University School of Medicine - Department of Biostatistics and Health Data Science, Indianapolis, IN*
[2] *Indiana University School of Medicine, Indianapolis, IN*
[3] *Regenstrief Institute - Center for Health Services Research*

Email: *jenrwest@regenstrief.org*

Background & Purpose

Testicular cancer (TC) is the most common solid malignancy diagnosed in men aged 14-40 years. Owing to both a relatively young age at diagnosis and a high curability rate (~95%), testicular cancer survivors (TCS) face a unique set of challenges involving their physical, mental, and emotional health – often lasting for decades after diagnosis. Identifying and addressing unmet supportive care needs in a timely manner is imperative. In PERSIST Phase 1, we assessed TCS' unmet needs/concerns and fear of cancer recurrence (FCR).

Methods

Between November and December 2024, 126 TCS were recruited from the Indiana University Simon Comprehensive Cancer Center to complete a one-time survey. Eligible participants were at least 18 years old, had completed TC treatment >2 weeks but ≤5 years before enrollment, and showed no evidence of active disease. Validated measures were used to assess unmet needs/concerns (the 24-item unmet needs/concerns scale from the National Coalition of Cancer Survivorship) and FCR (FCR-7). Associations between the number of needs/concerns reported by TCS and (i) time from treatment completion, (ii) age, and (iii) household income were assessed using the Spearman correlation.

Results

Participants' mean age was 31.1 years (*SD*=5.6). Participants were predominately white (90.4%) and non-Hispanic (93.1%), college-educated (64.1%), and employed full-time (79.5%). Findings revealed that 96.6% of participants reported having at least one unmet need/concern and 71.8% reported having 10 or more unmet needs/concerns. Of 24 needs/concerns assessed, participants reported 13.8 (*SD*=7.0) needs/concerns on average, with the top-rated concerns being (i) financial support, (ii) long-term planning/career goals, (iii) uncertainty about the future, (iv) maintaining a proper diet, and (v) family planning/fertility. Participants who completed treatment within the past 2 years had significantly more needs/concerns than participants who completed treatment 2-5 years ago (*P*=0.0149). Increased age (*P*=0.0305) and household income (*P*=0.0108) were negatively associated with the number of needs/concerns. Additionally, 49.6% of participants reported experiencing clinical FCR (defined by an FCR-7 score >17).

Conclusions

Despite a high curability rate, most TCS report unmet supportive care needs/concerns and many struggle with FCR. Future PERSIST study phases will employ participatory co-design to develop and then test an intervention targeting TCS' needs, including FCR in a pilot randomized controlled study.

Behavioral Research Technician

POSTER #88

THE IMPACT OF OBESITY, SOCIOECONOMIC BARRIERS, AND HEALTHCARE ACCESS ON ENDOMETRIAL CANCER RISK IN RURAL AND URBAN COMMUNITIES

Tessa Wrightson[1], Robert Miller[2]

[1] *Marian University Wood College of Osteopathic Medicine*
[2]*IU School of Medicine*

Email: *twrightson179@marian.edu*

Background: Like many communities facing significant healthcare disparities, endometrial cancer is more common in Appalachian communities than the US average. This is mainly due to factors like limited access to healthy food, high rates of obesity, and difficulty getting proper healthcare. Similar issues are seen in urban areas like Indianapolis, where people also face challenges that increase cancer risk. Although rural and urban areas might seem very different, both have neighborhoods that lack essential services and face barriers to getting affordable healthcare. Understanding these shared challenges is key to finding ways to reduce cancer risk in underserved communities.

Methods: To better understand these differences in Appalachia, we looked at data from the Kentucky Cancer Registry, an online database of cancer incidence and mortality. We specifically studied the number of endometrial cancer cases and deaths in Appalachian Kentucky and Non-Appalachian Kentucky over the past 20 years.

Results: Our research found that endometrial cancer rates were much higher in Appalachian Kentucky than in Non-Appalachian Kentucky. The number of cancer cases also went up in both regions over time. Additionally, mortality rose over the period of time studied, contrary to trends in other major cancer types across the United States.

Conclusion: The higher rates of endometrial cancer in Appalachian Kentucky show how lack of resources affect cancer risk in struggling communities. People in low-income areas, whether rural or urban, often have trouble accessing healthy foods and proper healthcare, which leads to higher obesity rates. Many of these communities face food insecurities, where fresh, healthy food is hard to find, and people rely more on processed, unhealthy foods. Being obese raises the amount of the hormone estrogen in the body, which increases the risk of endometrial cancer. Lack of healthcare access worsens outcomes, as people may not get regular check-ups or timely care for weight-related health problems. Solutions should aim to focus on teaching people about health, making healthy food more affordable and available, and encouraging exercise. Early efforts to prevent obesity, especially in young people, could help lower cancer risks in both rural and urban areas. If changes are not made, these health gaps will continue leading to higher cancer rates in underserved communities.

Community friendly research poster ***Medical Student***

POSTER #89

REFLECTIONS FROM THE INAUGURAL IUSCCC-AMERICAN CANCER SOCIETY POST-BACCALAUREATE PROGRAM (IADEP) COHORT

Keely Smith[1], Anaiya Crowner[1], Steven Verhagen[2], Rachel Bauer[2], Ann Kimble-Hill[3,4]

[1] *Indiana University Simon Comprehensive Cancer Center American Cancer Society Post-Baccalaureate Diversity In Cancer Research Education Program, Indianapolis, IN*
[2] *Indiana University Simon Comprehensive Cancer Center*
[3] *Department of Biochemistry and Molecular Biology, IU School of Medicine, Indianapolis IN*
[4] *Office of Inclusive Excellence, IU Simon Comprehensive Cancer Center*

Email: *smithkek@iu.edu*

The Indiana University Simon Comprehensive Cancer Center (IUSCCC) Post-Baccalaureate Program (IADEP) is an initiative designed to increase the representation of individuals from underserved communities within the cancer research and care workforce. IADEP aims to prepare fellows for successful entry into rigorous doctoral programs in biomedically relevant fields through a comprehensive two-year experience. Fellows engage in graduate entrance exampreparation, responsible conduct of research training, professional development, and cancer research experiences to build foundational career competencies. Those with clinically focused aspirations are further supported through structured shadowing opportunities, Medical College Admissions Test (MCAT) preparation, and engagement with IU School of Medicine affinity groups. Ongoing individualized support from the IU Indianapolis Graduate School, IU School of Medicine, and IUSCCC ensures skill development, application readiness, and resilience in academic environments. The programmatic goal is for at least 75% of Fellows to matriculate into competitive graduate programs.This poster showcases highlights, successes and memories from the inaugural cohortas their time in the program comes to a close. The work shared highlights the benefits and areas of opportunity for future cohorts within the IUSCCC ecosystem.

Community friendly research poster ***Post-Baccalaureate (IADEP)***

POSTER #90

REFLECTIONS FROM THE INAUGURAL IUSCCC-AMERICAN CANCER SOCIETY POST-BACCALAUREATE PROGRAM (IADEP) COHORT

Anaiya Crowner [1], Keely Smith [1], Steven Verhagen[1], Rachel Bauer[1], Dr. Anne Kimble-Hill[1,2]

[1] *Indiana University Melvin and Bren Simon Comprehensive Cancer Center*
[2] *Indiana University School of Medicine*

Email: *acrowner@iu.edu*

The Indiana University Simon Comprehensive Cancer Center (IUSCCC) Post-Baccalaureate Program (IADEP) is an initiative designed to increase the representation of individuals from underserved communities within the cancer research and care workforce. IADEP aims to prepare fellows for successful entry into rigorous doctoral programs in biomedically relevant fields through a comprehensive two-year experience. Fellows engage in graduate entrance exam preparation, responsible conduct of research training, professional development, and cancer research experiences to build foundational career competencies. Those with clinically focused aspirations are further supported through structured shadowing opportunities, Medical College Admissions Test (MCAT) preparation, and engagement with IU School of Medicine affinity groups. Ongoing individualized support from the IU Indianapolis Graduate School, IU School of Medicine, and IUSCCC ensures skill development, application readiness, and resilience in academic environments. The programmatic goal is for at least 75% of fellows to matriculate into competitive graduate programs. This poster showcases highlights, successes and memories from the inaugural cohort as they their time in the program comes to a close. The work shared highlights the benefits and areas of opportunity for future cohorts within the IUSCCC ecosystem.

Community friendly research poster ***Post-baccalaureate***

POSTER #91

WHAT I WISH I KNEW ABOUT THE SURGICAL OPTIONS FOR BONE CANCER: "FUNCTION IS MORE IMPORTANT THAN LOOKS!"

Janet Panoch[3], Clayton Hicks[1], Christopher Collier[2]

[1] *IU School of Medicine, Indianapolis, IN*
[2] *Department of Orthopaedic Surgery, IU School of Medicine, Indianapolis, IN*
[3] *Walther Supportive Oncology Program*

Email: *jpanoch@iu.edu*

Background. Osteosarcoma and Ewing sarcoma rare, primarily pediatric bone cancers with about 1200 cases per year in the United States. Most tumors are in the leg bones with surgical options that may include amputation, limb salvage, or rotationplasty. Families and patients may be engaged in shared decision making with their surgeon since the risk for cancer coming back is the same. Little is known about the patient's experience of life after surgery.

Methods. Eligible participants were over age 18, diagnosed with osteosarcoma or Ewing sarcoma or be a caregiver/family member. A Survey Monkey survey was shared to four Osteosarcoma/Ewing sarcoma Facebook support groups with three questions that included When you think about life after surgery, what do you wish you knew? Any names in the responses were changed so it was anonymous, then analyzed by two researchers to explore life after surgery for people with bone cancer.

Results. After four weeks, there were 155 responses, mostly parents (n=99. Most of the participants were under the age of 18 when they had their first surgery (n=123) with cancer in the leg (n=134). More than half of the participants (n=86) said their first surgery was limb salvage. Time since the first surgery ranged from two weeks to 40 years.

For participants who had limb salvage, nearly all wish they knew what they could not do. They also wish they knew about common problems (infection, implant loosening, and chronic pain) and the need for more surgeries. They wish they knew how long the limb salvage would last and that each surgery had "diminishing returns" for success. Some participants chose to have an amputation or rotationplasty after limb salvage because of these issues.

For those who had amputation or rotationplasty, they wish they knew about the cost and process of getting fitted for artificial legs, especially for a growing child.

For all participants, they wish options were explained better with more focus on what they could do. Regrets were about having a surgery that did not let them do what matters most to them.

I wish I knew about the limited mobility I would have. I wish I knew that amputation may be a benefit and not a loss. I wish I would've known that I would be electing for amputation at 30 years old. I wish I knew that "at least I still have my leg" isn't the win against cancer that I wanted. – patient who had limb salvage at age 14

Conclusion. These findings suggest that patients and families feel unprepared for life after surgery. They regret not having options or not being informed about what they could or could not do. Patient education is needed for making this important surgical decision.

Community friendly research poster ***Research Technician***

POSTER #93

HEALTH AND BREAST CANCER AWARENESS AMONG ADULTS AT THE DOMINICAN-HAITIAN BORDER: A PILOT STUDY

Emma Dellinger[2], Juwaiyrriyah Omar[1], Amy Obringer[1]

[1] *University of Saint Francis, Fort Wayne, IN*
[2] *University of Saint Francis*

Email: *emma.dellinger@gmail.com*

While breast cancer mortality rates have decreased in developed countries, low- and middle-income countries are experiencing risingbreast cancer deaths. In the clinic in Rosa La Piedra—a mountain village located in the Dominican Republic near the border of Haiti— women with breast cancer often present with very advanced disease, a trend attributed to significant socioeconomic and educational challenges. Delays in seeking medical care have been linked to limited resources, cultural beliefs, geographical constraints, low levels of health literacy, and stigma.

A pilot study was initiated to assess existing knowledge, health perceptions, and obstacles related to breast cancer awareness among adults in Rosa La Piedra. The study employs a cross-sectional survey administered verbally to overcome literacy barriers. Survey items focus on awareness of breast cancer risk factors, recognition of early signs, understanding of disease etiology, and perceived obstacles to seeking timely care. Volunteer medical teams administered the survey during two brief trips to Rosa La Piedra in May and November of 2024. A total of 24 surveys were completed. The findings of these surveys support the initial assessment that there are critical knowledge gaps and misconceptions about breast cancer. For instance, of the 18 participants who were asked, “what do you think causes breast cancer?” Ten participants did not know, seven participants believed that an injury to the breast caused the cancer, and one believed that the cancer occurred from not taking their medication.

The results of this study have provided valuable information on the local beliefs and challenges of Rosa La Piedra that can delay medical care. These insights and additional results collected as the survey continues to be administered will be used to develop targeted educational interventions and support strategies, such as establishing a medical fund to facilitate access to diagnostic and treatment services. Increasing breast cancer awareness in underserved communities worldwide, such as Rosa La Piedra, is essential to promoting early detection and preventing the rising rates of both incidence and mortality of breast cancer.

Community friendly research poster ***Undergraduate Student***

POSTER #94

THE ASSOCIATION BETWEEN CERVICAL CANCER TREATMENT AND SURVIVAL TIMES IN A POPULATION OF CERVICAL CANCER PATIENTS IN THE DEMOCRATIC REPUBLIC OF THE CONGO: A RETROSPECTIVE COHORT STUDY.

Jonas Ndeke[1], Jonathan T Macy[2], James E Klaunig[3], Antoine M Mbutuku[4], John K Mufuansoni[4], Bismark M Mpembele[5], Lievain D Lukuaka[6], Douglas Landsittel[1,7]

[1] Department of Epidemiology and Biostatistics, Indiana University School of Public Health, Bloomington, IN, USA
[2] Department of Applied Health Science, Indiana University School of Public Health, Bloomington, IN, USA
[3] Department of Environmental and Occupational Health, Indiana University School of Public Health, Bloomington, IN, USA
[4] Department of Obstetrics and Gynecology, Hôpital Général, Kinshasa, Democratic Republic of the Congo
[5] Department of Obstetrics and Gynecology, Hôpital Général de Référence Institut Médical Evangélique (IME) de Kimpese, Kimpese, Kongo Central Province, Democratic Republic of the Congo
[6] Hôpital Général de Référence Institut Médical Evangélique (IME) de Kimpese, Kimpese, Kongo Central Province, Democratic Republic of the Congo
[7] Department of Biostatistics, University at Buffalo School of Public Health and Health Professions, Buffalo, NY, USA

Email: *jndeke@iu.edu*

BACKGROUND: With nationally limited curative-intent treatments, cervical cancer is the leading cancer in terms of mortality in the Democratic Republic of Congo (DRC). No prior study has assessed the association between treatment received and overall survival in patients in the DRC.

METHODS: We retrospectively reviewed patient data from 2014-2023 in two DRC hospitals, Hospital General (HG, urban) and IME Kimpese (IME, rural). Type of treatment received (curative-intent, palliative/none) was the exposure, and life status was the outcome. Covariates included cancer stage. We completed missingness, descriptive, and bivariate (t-test and chi-square, at a= .05) analyses. We specified Kaplan-Meier curves and, after checking assumptions, a Cox proportional hazard model to estimate hazard ratios (HR).

RESULTS: The 423/732 cases (265 at HG, 158 at IME) and 101/240 deaths included in the full analysis totaled 262.6 person-yrs, with baseline mean (SD) age of 53.4 (12.0) yrs and median (IQR) follow-up time at 0.18 (0.05, 0.62) yrs. Overall, 279 (65.6%) were late-stage (IIb-IVb) cancers (77.0% at HG, 47.5% at IME; p-value <.0001), 230 (54.4%) received a curative-intent treatment (43.0% at HG, 73.4% at IME; p-value <.0001). Kaplan-Meier curves differed across settings and diagnosis period. Curative-intent treatment reduced crude hazard of mortality by 89%/71%, respectively at HG, HR (95% CI) 0.11(0.06, 0.21), and IME (0.29 [0.13, 0.63]). Adjusting for the diagnosis period (ref. 2018 or earlier), this protective effect was at 86%/69%, respectively, with adjusted HR (95% CI) 0.14 (0.07, 0.26) for HG and 0.31 (0.14, 0.68) for IME.

CONCLUSION: In this first ever survival study reported for the DRC, most cases were difficult to track, diagnosed at a late stage, and with very low overall survival, as in most of sub-Saharan Africa. However, receiving any curative-intent treatment was associated with relatively better survival. Further investigation with nationally representative data is warranted.

Key words: cervical cancer survival, cervical cancer treatment, low-resource settings, global health, Democratic Republic of Congo, sub-Saharan Africa

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

POSTER #95

RELATIONSHIP BETWEEN NEIGHBORHOOD-LEVEL SOCIOECONOMIC STATUS AND RELAPSED PEDIATRIC B-ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH CAR-T19 THERAPY IN INDIANA

Jinan Ayub[3], Alyssa Walker[1], Tyra Grischke[1], Gloria Garcia[1], Jodi Skiles[2], Courtney Spiegel[2], Sandeep Batra[2]

[1] *Indiana University School of Medicine, Indianapolis, IN*
[2] *Department of Hematology-Oncology, Riley Hospital for Children, Indianapolis, IN*
[3] *Indiana University School of Medicine*

Email: *jmayub@iu.edu*

Background/Purpose:

There is paucity of studies investigating the relationship between socioeconomic status and outcomes for patients receiving chimeric antigen receptor T-cell therapy (CART19) for refractory or relapsed B-ALL. Area Deprivation Index (ADI) serves as a measurement of socioeconomic disadvantage based on theoretical income, education, employment, and quality of housing.

Methods:

A retrospective chart review of 26 patients with relapsed B-ALL treated with CART19 at a non-profit children's hospital from 2018-2024 was conducted. Using a public institution's Neighborhood Atlas database, ADI scores (range 1-10) were recorded for each patient based on ZIP code of residence in Indiana. A low ADI score (0-5) indicates affluence and higher SES, while a high ADI score (6-10) indicates deprivation and a lower SES. Comparisons between groups were done using Chi-square tests for categorical variables. The Kaplan-Meier method was used to analyze relapse free survival (RFS) using the log rank test to compare groups.

Results:

The patients (age range 2-25 years) were stratified into two groups: low ADI or high SES (≤5, n = 10 (37%); 2.9 ± 1.4; range, 1-5)) and high ADI or low SES (>5, n = 17 (63%); 8.5 ± 1.5; range, 6-10). Seventeen identified as white (63%) and 10 (37%) as Hispanic. Three patients received CART19 infusions twice, and one patient received multiple CART19-directed products. In the high ADI group, 3-month, 6-month, and 1-year RFS post-CART therapy was 82%, 76%, 60% respectively compared to 100%, 71%, 57% in the low ADI group (p=0.9). In the high ADI group, 47% experienced toxicities from CAR-T19 compared to 44% in the low ADI group ($X2(1) = 0.0162$, $p = 0.90$). In the high ADI group, 41% had pre-existing co-morbidities compared to 56% in the low ADI group ($X2(1) = 0.4896$, $p = 0.48$).

Conclusion:

Most patients who received CART therapy at our tertiary center resided in low SES areas but did not experience worse RFS, increased toxicities from CAR-T19, or suffer from more pre-existing co-morbidities compared to patients residing in more affluent areas. Further studies with larger sample sizes are needed to better understand the heath inequalities among patients with relapsed leukemia in Indiana and to identify challenges faced by patients from disadvantaged communities with limited resources.

Population Science/Epidemiology ***Medical Student***

POSTER #96

THE IMPACT OF OBESITY, SOCIOECONOMIC BARRIERS, AND HEALTHCARE ACCESS ON ENDOMETRIAL CANCER RISK IN RURAL VERSUS URBAN COMMUNITIES

Tessa Wrightson[1], Robert Miller[2]

[1] *Marian University Wood College of Osteopathic Medicine*
[2]*IU School of Medicine*

Email: *twrightson179@marian.edu*

Background: Endometrial cancer disproportionately affects the Appalachian population compared to the overall United States population. This disparity is largely attributed to health inequities, including limited access to nutritious foods, high obesity rates, and metabolic syndromes, alongside disparities in healthcare access. Similar trends have been observed in urban populations, such as Indianapolis, where socioeconomic barriers contribute to increased cancer incidence. While rural and urban populations may seem distinct, both face structural healthcare challenges that exacerbate cancer disparities. Understanding these shared struggles is crucial for creating effective public health strategies that can reduce cancer risk in underserved communities, no matter the location, furthermore, developing effective public health interventions that address cancer risk factors in diverse, underserved communities.

Methods: To explore this issue, we analyzed data from the Kentucky Cancer Registry's online Cancer Incidence and Mortality Registry to assess trends in age-adjusted invasive corpus uteri cancer incidence and mortality in Appalachian Kentucky (AK) and Non-Appalachian Kentucky (NAK) counties from 2001 to 2020. Incidence rate ratios were used to compare cancer incidence and mortality between the two regions across multiple time periods, and the z-test for two population proportions was applied to evaluate statistical significance.

Results:

Year	Incidence AK (/100,000)	Incidence NAK (/100,000)	% Higher Incidence AK	Mortality AK (/100,000)	Mortality NAK (/100,000)	% Higher Mortality AK
2001-2005	25.3	21.0	20.5%	1.6	1.6	0.0%
2006-2010	27.2	22.1	23.1%	1.5	1.6	-6.3%
2011-2015	30.3	24.7	22.7%	2.0	1.7	17.6%
2016-2020	31.8	26.0	22.3%	2.5	2.6	-3.8%

Endometrial cancer incidence was significantly higher in AK than in NAK at the beginning ($P<0.002$) and end ($P<0.001$) of the study period. However, mortality differences between the two regions were not statistically significant. Both incidence and mortality increased over time in AK ($P<0.01$) and NAK ($P<0.001$).

Conclusion: The consistently higher incidence of endometrial cancer in Appalachian Kentucky reflects the impact of socioeconomic and healthcare disparities. Lower socioeconomic status is linked to restricted access to nutritious foods and healthcare, contributing to a rise in chronic conditions like obesity. This, in turn, increases exposure to unopposed estrogen, heightening the risk of endometrial cancer, as reflected in these findings. While these disparities are often attributed to the region's unique landscape, they mirror broader challenges faced in urban areas, such as Indianapolis, where similar

socioeconomic barriers drive cancer incidence. Addressing these disparities requires targeted interventions, including improving health literacy, increasing access to affordable nutritious foods, and promoting physical activity. Early prevention efforts, especially among adolescents, may help mitigate obesity-related cancer risks and reduce long-term disease burden in both rural and urban populations.

Population Science/Epidemiology ***Medical Student***

POSTER #97

IMPACT OF PATIENT TRAVEL AND CARE FRAGMENTATION ON RECEIPT OF CHEMOTHERAPY AND CLINICAL OUTCOMES AFTER PANCREATECTOMY FOR PANCREATIC CANCER

Alexa Hughes[2], Kristen Kaiser[1], Brian Ruedinger[2], Anita Turk[3], Thomas Maatman[4], Michael House[4], Karl Bilimoria[2], Ryan Ellis[2]

[1] *Surgical Outcomes and Quality Improvement Center (SOQIC), Department of Surgery, Indiana University School of Medicine, Indianapolis, IN*

[2] *Surgical Outcomes and Quality Improvement Center (SOQIC), Department of Surgery, Indiana University School of Medicine*

[3] *Division of Hematology & Oncology, Department of Medicine, Indiana University School of Medicine*

[4] *Division of Surgical Oncology, Department of Surgery, Indiana University School of Medicine*

Email: *alhughe@iu.edu*

Background: While high-volume hospitals(HVHs) achieve superior surgical outcomes for pancreatic ductal adenocarcinoma (PDAC) resections, the impact of travel distance and fragmentation of care on adherence to guideline-concordant adjuvant chemotherapy and survivalremainsunclear. Theobjectives of this study were to (1) evaluate the associations between travel distance and care fragmentation on thereceipt of adjuvant chemotherapy in patients undergoing upfront resection for PDAC at HVHs and (2) examine how these factors influence survival.

Methods:The National Cancer Database (2007–2021) was retrospectively queried for patients diagnosed with PDAC who underwent upfront surgical resection at anHVH. The cohort was stratified based on receipt of adjuvant chemotherapy, travel distance in deciles (D1–D10), and care coordination status—categorized as coordinated care (treatment at a single facility) or fragmented care (treatment at multiple facilities). Multivariable regression analysis was performed to identify factors associated with the receipt of chemotherapy and fragmented care. Survival outcomes were assessed using Cox proportional hazards models and Kaplan-Meier analysis.

Results:A total of17,807 patients from 97 HVHs were included in analysis.Most patientswere male (52.0%) andhad stage II disease (77.2%). Patients traveling more than16 miles were less likely to receive adjuvant chemotherapy (D4, 16 miles, 63.4%, OR 0.85, 95% 0.73-0.99, P=0.04), with chemotherapy rates decreasing as travel distance increased(Table 1). Patients who receivedfragmented care were more likely to receive adjuvant therapy (64.3%, OR 1.51, 95% CI 1.35-1.69, p<0.001). Patients traveling more than 22 miles had a higher risk of mortality (HR 1.12, 95% CI 1.02-1.23, P= 0.01), with mortality riskincreasing progressively with travel distance. However, patients who received adjuvant chemotherapy (HR 0.77, 95% CI 0.73-0.81, P <0.001) and those with fragmented care (HR 0.89, 95% CI 0.84-0.93, P<0.001) had a lower risk ofmortality.

Conclusions: Patients undergoing resection for PDAC at an HVH were less likely to receive adjuvant chemotherapy if they traveled further, while patients with fragmented care had higher adjuvant therapy rates and improved mortality. As surgical care centralizes to HVHs, enhancing multidisciplinary coordination within and across health systems may improve multimodal care and outcomes for patients traveling for complex surgery.

Table 1: Odds of Receipt of Adjuvant Chemotherapy in the OverallCohort

Parameter	Rate (%)	OR (95% CI)	P-value
Distancein deciles (average ± SD in miles)			
D1 (3 ± 1)	62.7%	ref	ref
D2 (7 ± 1)	63.7%	1.00 (0.87-1.14)	0.99
D3 (11 ± 1)	64.2%	0.96 (0.83-1.12)	0.62
D4 (16 ± 2)	63.4%	0.85 (0.73-0.99)	0.04
D5 (22 ± 2)	61.5%	0.78 (0.66-0.91)	0.002

D6 (30 ± 3)	56.7%	0.65 (0.55-0.77)	<0.001
D7 (42 ± 4)	55.8%	0.65 (0.56-0.76)	<0.001
D8 (60 ± 7)	53.7%	0.61 (0.52-0.73)	<0.001
D9 (91 ± 12)	47.5%	0.50 (0.41-0.61)	<0.001
D10 (162 ± 36)	43.4%	0.40 (0.32-0.50)	<0.001
Fragmented Care			
No	54.4%	ref	ref
Yes	64.3%	1.51 (1.35-1.69)	<0.001

POSTER #98

EVIDENCE OF HPV 16 VIRAL LATENCY IN CLOSELY FOLLOWED UNVACCINATED ADOLESCENT WOMEN

Teresa Imburgia[1,4], Michele Cote[2], Aaron Ermel[3]

[1] Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Vera Bradley Scholar, Indianapolis, IN
[2] Komen Tissue Bank at Indiana University Simon Comprehensive Cancer Center, Department of Epidemiology, Indiana University Richard M. Fairbanks School of Public Health, Indianapolis, IN
[3] Division Infectious Diseases, Indiana University School of Medicine, Indianapolis, IN
[4] Department of Epidemiology, Indiana University Richard M. Fairbanks School of Public Health

Email: *tcumming@iu.edu*

Background: HPV natural history has two critical areas of uncertainty that can impact how we prevent cervical cancer: 1) Whether natural HPV infection protects against reinfection of the same HPV type and 2) Whether the loss of HPV detection reflects clearance or viral latency, i.e. whether episodic re-detection of oncogenic HPV in women represents viral re-activation from a latent state or is representative of a newly acquired infection. Re-activation implies that women are still at risk for HPV-related cancer outcomes later in life even after the virus is not detectable in clinical samples. In this study, we demonstrate periods of episodic detection of HPV 16 with and without the presence of sexual activity in a closely followed unvaccinated cohort from high STI communities.

Methods: Data were from 12 women who were a part of a cohort of unvaccinated adolescents (total n=387) recruited at ages 14-17 beginning in 1999 and followed for approximately 6.4 years. Quarterly interviews and vaginal swabs along with 6 months of daily sex behavior diaries with corresponding weekly vaginal swabs were available. The 12 participants in our study had vaginal swabs tested for HPV at each of their quarterly visits using the Roche Linear Array in past studies. All 12 participants had a positive HPV 16 detection, followed by a period of non-detection, with a three-month daily diary and weekly swab collection leading up to a re-detection of HPV 16. We utilized the BD Onclarity™ HPV Assay on frozen samples of archived weekly vaginal swabs and compared them with the daily sex diaries.

Results: The 12 women were in the original cohort for an average of 6.4 years (range 4.2-9.0). Re-detection of HPV 16 occurred around 4 years (m=4.08) with the first HPV 16 detection towards the end of the first year (m=1.75). During three months of weekly sampling, 5 were episodic in their detection, 4 were continuously positive once re-detection occurred, and 3 were HPV 16 negative until their final swab in the collection period. For the 5 participants with episodic HPV 16, the re-detection events (n=22) were not associated with sexual encounters. All those with episodic detection went on to have either low-grade or high-grade cervical lesions within ten years of the collection period. None of the 3 women positive only at their final swab had reported sex in the seven days leading up to the sampling.

Conclusion: HPV, when sampled at short intervals, can be episodic regardless of sexual encounters. We provide evidence of viral latency where no sexual activity takes place before the sampling, yet the participant has HPV 16 re-detected. These data provide evidence that after two negative test results, women may still be at risk for cancer progression in the future.

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

POSTER #99

POOR MENTAL HEALTH DAYS AND DEPRESSIVE DISORDERS BY INFORMAL CANCER PATIENT CAREGIVER STATUS: BRFSS 2022-23

Jeanne Ward[3], Victoria Champion[1], John Blosnih[2]

[1] *School of Nursing, Indianapolis, IN*
[2] *Suzanne Dworak-Peck School of Social Work, Los Angeles, CA*
[3] *School of Nursing*

Email: *jeanneward@iu.edu*

Background: Informal caregivers (ICs) of cancer patients substantially influence patient outcomes, but their mental health needs are understudied. Limited data show that ICs of cancer patients may have mental health needs exceeding those of the general population.

Aims: This analysis sought to compare correlates of socio-demographic and physical factors related to these ICs' mental health.

Methods: Data from the Behavioral Risk Factor Surveillance Survey (BRFSS) 2022-23 caregiving module were analyzed to investigate correlates predicting poor mental health and depression, comparing ICs to the general population. Analyses examined associations for each correlate. Separate logistic regressions identified factors associated with six or more poor mental health days monthly, 14 or more poor mental health days monthly, or depression. Predictors included reporting 14 or more poor physical health days monthly, age, sex, marital status, education, employment status, income, and rural/urban status.

Results: Twenty-four states administered the caregiving module, including 199,664 individuals, with 3,028 identifying as ICs to cancer patients. In unadjusted models, ICs were significantly more likely than the general population to be older, female, White, married/partnered, have attended college, be nonemployed, earn less than $50,000 annually, live in a rural location, and report more poor physical and mental health days and depression. In adjusted models, ICs, compared to the general population, were significantly more likely to report six or more poor mental health days per month.

Conclusions: Future research should address risk factors associated with poor mental health in ICs of cancer patients, identifying the most at-risk ICs for targeted interventions.

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

POSTER #100

GEOECOLOGICAL RISK ASSESSMENT OF AGGRESSIVE MENINGIOMAS IDENTIFIED IN A RURAL HEALTH SYSTEM IN PENNSYLVANIA

Timothy Caldwell

Email: *timcaldw@iu.edu*

Meningiomas are the most common intracranial tumor. These tumors emerge from arachnoid cells. These tumors are traditionally treated with gross total resection. Current risk factors leading to carcinogenesis include ionizing radiation and hormonal exposure. Women have an increased incidence of meningiomas due to these tumors expressing a greater density of estrogen and progesterone receptors. Recently, it has been demonstrated that pesticide exposure has contributed towards tumorigenesis however there continues to be a substantial gap in identifying risk factors leading to the development in meningiomas. To that end, we aimed to determine geoecological factors contributing to the incidence of meningiomas in rural Pennsylvania. Methods: We retrospectively evaluated 415 pathology confirmed WHO Grades I-III meningioma cases within the Geisinger Health system. Cases were geocoded and mapped by county prior to stratification by incidence, tumor grade, and tumor aggressive behavior. GraphPad Prism 8 was utilized to quantify percentiles of meningioma cases. Results. Our work demonstrated that the highest incidence of meningiomas were localized to the Lackawanna and Luzerne counties irrespective of WHO classification. Further analyses highlight that female patients had a higher incidence of cases than males. Incidence was also correlated to Lackawanna and Luzerne counties. 303 WHO grade I patients were later reassessed for disease recurrence and showed a higher incidence along Luzerne, Columbia and Montour counties. Finally, meningioma aggressiveness was assessed. Our work shows that males have a 12% greater risk of developing a meningioma that is more aggressive. We further investigated these findings to assess the severity of WHO grade meningioma that can develop by sex and concluded that males ages 51-60 are more likely to develop a WHO Grade II or III meningioma whereas males aged 61-70 are more likely to have a WHO Grade I meningioma. In contrast, younger females are more likely to have a WHO Grade I meningioma but as they age, the risk of developing a more aggressive meningioma was shown to increase. Conclusions. Our large population demonstrated that a higher incidence of meningiomas and meningioma recurrences are localized towards Pennsylvania counties bordering the Lackawanna River supply. Our work also demonstrated that male patients had a 12% higher risk of developing meningiomas that were more aggressive.

Population Science/Epidemiology ***Research Technician***

POSTER #101

FOODRX: INTEGRATING NUTRITION INTO CANCER CARE, USABILITY AND VALIDATION OF THE DIET ID™ TOOL

Jocelyn Yang[1], Daniel Clark[2,3], Richard Holden[1,2], Titus Schleyer[2,3], Rebecca Rivera[2,3]

[1] *Indiana University, Bloomington, IN*
[2] *Regenstrief Institute, Inc., Indianapolis, IN*
[3] *Indiana University School of Medicine, Indianapolis, IN*

Email: *joceyang@iu.edu*

Background: One in four cancer survivors faces food insecurity and heightened risk of poor nutrition. Proper nutrition is crucial for cancer care, making this a significant healthcare and health equity issue. Within our prior pilot study, a major theme advanced practice providers emphasized was patients' desire for nutrition guidance.

Objective: To evaluate the acceptability, validity, and reliability of the Diet-ID™ tool among oncology patients and providers Indiana University Simon Comprehensive Cancer Center (IUSCCC).

Methods: The planned study will enroll adult cancer survivors who have undergone curative-intent therapy in the past 12 months at downtown Indianapolis and Schwarz in Carmel IUSCCC sites.

Developed by Dr. David Katz, Diet-ID™ uses Diet Quality Photo Navigation, a validated approach to diet assessment based on visual pattern recognition. Participants select from a series of images that best represent their typical diet until the best possible fit is achieved, rapidly identifying baseline dietary patterns and scoring diet quality within minutes. Diet ID™ derives diet quality scores from the Healthy Eating Index (HEI-2020). Audience demos and feedback will be encouraged during the IUSCCC Cancer Research Day.

We will evaluate the acceptability of Diet-ID™ among cancer care stakeholders. Patients (n=200) will complete Diet-ID™ and validated technology acceptance model (TAM) surveys during routine cancer surveillance appointments. Feedback will be gathered through surveys and semi-structured interviews with patients (n=20) and providers (n=20) to understand their perceptions and attitudes of integrating diet assessment in the clinic using Diet-ID™. Qualitative data will be analyzed using a rapid qualitative analytic approach with deductive analysis mapping data to TAM constructs to develop themes. Validity will be evaluated against mean usual dietary intake calculated using up to two administrations of the Automated Self-Administered 24-Hour Dietary Recall tool (ASA24®), the gold standard for dietary assessment created by the National Cancer Institute. Test-retest reliability of Diet-ID™ will be evaluated with two repeat administrations within one week.

Diet quality will be explored quantitatively for potential differences between groups based on characteristics, with special emphasis on groups at risk of health inequities. Data collection is ongoing.

Conclusion: This study builds the foundation for leveraging health informatics and dietary data resources in support of cancer survivors' equitable access to health-promoting-foods and nutrition in cancer care.

Population Science/Epidemiology ***Undergraduate Student***

POSTER #102

AI-BASED ROBUST TESTICULAR CANCER TRIAGING, A PROMISE FOR AN EFFICIENT AND COST-EFFECTIVE DIAGNOSTIC MODALITY

Siddhesh Thakur[1]

[1] *Department of Pathology and Laboratory Medicine*

Email: *thakursp@iu.edu*

Background: Testicular cancer is one of the most common malignancies in males aged 20-44, with its two major subtypes being Seminoma and Non-Seminomatous Germ Cell Tumors (NSGCT). Accurate classification is essential for patient management and treatment, after initial histologic diagnosis on radical orchiectomy specimens or biopsies of metastases. Histopathological diagnosis, though effective, is time-consuming, costly, and subject to inter-observer variability. Digital pathology combined with artificial intelligence (AI) promises to improve diagnostic consistency and efficiency while benefitting settings without expertise in these tumors. We seek rapid and robust triaging of these subtypes through a foundation model interrogating H&E-stained tissue sections and an attention-based Multiple Instance Learning (abMIL) approach providing interpretable predictions highlighting decision-driving regions.

Design: Retrospective multi-institutional data from 144 patients (254 slides) were used to develop our AI model. 118 patients (202 slides) were used for model training, 14 (30 slides) for validation, and 14 (22 slides) as an independent hold-out set. The UNI foundation model was pre-trained on diverse datasets and fine-tuned using abMIL, identifying discriminative features from the training data without requiring pixel-level annotations.

Results: Independent hold-out set evaluation revealed Area Under the Curve=96%, Accuracy=94.54%, Specificity=92%, and importantly, Sensitivity=100%. Specifically, all predicted NSGCT are correctly identified as NSGCT, and all Seminomas are always detected. In other words, no Seminoma case is overlooked, while 8% of the actual NSGCT may be misclassified as Seminoma. A sensitivity of 100% supports our model as a triaging tool, enabling faster decision-making for high-risk patients.

Conclusion: Our proposed abMIL approach leveraging the foundation model UNI, provides a perfectly sensitive and scalable solution for clinical triaging between Seminoma and NSGCT testicular tumors in digitized tissue sections, ensuring subtype detection and supporting pathologists in improving diagnostic accuracy and efficiency. Furthermore, the model's focus on highlighting AI decision-driving regions of the tissue section, renders it well-suited for large-scale clinical data, where it can make further data-driven contributions in extending our current disease knowledge.

Translational/Clinical Research ***Data Engineer***

POSTER #103

COMBATING MPNST WITH DUAL METABOLIC INHIBITION: OXPHOS AND GLUTAMINOLYSIS AS KEY TARGETS

Silpa Gampala[1], Marisa Ciesielski[1], Chi Zhang[2], Steven Rhodes[1], Melissa Fishel[3]

[1] Department of Pediatrics and Herman B Wells Center for Pediatric Research, Indiana University Simon Comprehensive Cancer Center, Indiana University School of Medicine, Indianapolis, IN 46202, USA

[2] Indiana University Simon Comprehensive Cancer Center, Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN 46202, USA. Department of Biomedical Engineering, Oregon Health & Science University, Portland, OR 97239, USA

[3] Department of Pediatrics and Herman B Wells Center for Pediatric Research, Indiana University Simon Comprehensive Cancer Center, Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46202, USA

Email: *sgampala@iu.edu*

Malignant peripheral nerve sheath tumor (MPNST) is a rare and aggressive soft tissue sarcoma with poor prognosis and limited treatment options. Surgical resection remains the primary approach but is often ineffective due to tumor size, location, and high recurrence rates. Conventional chemotherapy, such as doxorubicin/ifosfamide, as well as targeted therapies using kinase inhibitors, have shown limited efficacy due to toxicity and the complexity of the tumor microenvironment. Loss of the **Nf1** (*Neurofibromatosis type I*) gene leads to sustained Ras activation and dysregulation of metabolic pathways, including glutaminolysis, oxidative phosphorylation (OXPHOS), and the tricarboxylic acid (TCA) cycle. Targeting these metabolic vulnerabilities offers a promising therapeutic strategy for MPNST. Our work demonstrates that **Redox Factor-1 (Ref-1)** regulates mitochondrial metabolism, and its inhibition downregulates mitochondrial gene expression and impairs the TCA cycle, forcing MPNST cells to rely on alternative metabolic pathways like glutaminolysis. Ref-1 is highly expressed in MPNST patient samples, with expression levels increasing as disease progresses toward malignancy. RNA-seq analysis revealed that Ref-1 inhibition reduces OXPHOS activity, potentially compensated by increased reliance on glutamine metabolism. Glutamine supports MPNST growth by fueling nucleotide synthesis and TCA cycle. **JHU395**, a prodrug of glutamine antagonist **DON**, has been shown to inhibit MPNST tumor growth by blocking glutamine-dependent purine biosynthesis. Building on these findings, ***we hypothesized that dual metabolic inhibition using the Ref-1 inhibitor APX2009 in combination with JHU395 would significantly impair two critical metabolic pathways, offering a novel therapeutic strategy for this lethal disease.***

Human MPNST cells exhibited significantly elevated basal metabolic demands, with increased glycolysis, OXPHOS activity, enhanced utilization of TCA cycle intermediates, and elevated expression of key metabolic proteins such as ACLY, IDH2, and NDUFS4, compared to immortalized normal Schwann cells. These cells also displayed heightened glutamine dependency. Treatment with APX2009 and JHU395 as single agents induced dose- and time-dependent cytotoxicity in MPNST cells, with further enhancement observed in combination. In vivo efficacy was evaluated using a high-penetrance genetically engineered mouse model (GEMM) of Nf1-deficient MPNST (***Nf1$^{flox/flox}$; Arf$^{flox/flox}$; PostnCre(+)***) to assess disease progression from plexiform neurofibromas (PNFs) to MPNST, and a syngeneic orthotopic model derived from ***Trp53^{tm1Tyj}Nf1^{tm1Tyj}* mice** (mNF463a cells) to evaluate tumor growth inhibition. These complementary models were used to determine whether metabolic inhibition could delay MPNST progression and/or provide therapeutic benefit in established tumors. Using these models, we observed improved survival in the disease progression model and significant tumor shrinkage in the syngeneic orthotopic model with combined inhibition of OXPHOS and glutaminolysis. *These findings underscore the critical role of metabolic reprogramming in MPNST and establish dual inhibition of Ref-1 and glutaminolysis as a promising therapeutic strategy. The observed tumor regression and survival benefit in preclinical models highlight the potential of this approach to delay disease progression and improve patient outcomes.*

Translational/Clinical Research ***Faculty***

POSTER #104

COMBINATION MVF-TIGIT-2/4 WITH PD1-VAXX, PDL1-VAXX AND CTLA-4 VAXX IN SYNGENEIC BALB/C MODELS CHALLENGED WITH CT26 (COLON), 4T1 (TNBC) AND D2F2 (BREAST).

Linlin Guo[3], Jay Overholser[1], Pravin Kaumaya[2]

[1] *Indiana University School of Medicine, Simon and Bren Comprehensive Cancer Center, Indianapolis, IN*
[2] *Indiana University School of Medicine, Department of Microbiology & Immunology, Indianapolis, IN*
[3] *Indiana University School of Medicine, Department of Microbiology & Immunology*

Email: *llinguo@iu.edu*

Combination MVF-TIGIT-2/4 with PD1-Vaxx, PDL1-Vaxx and CTLA-4 Vaxx in syngeneic BALB/c models challenged with CT26 (colon), 4T1 (TNBC) and D2F2 (breast).

Linlin Guo, Jay Overholser and Pravin T. P Kaumaya

Indiana University School of Medicine, Department of Microbiology & Immunology, Simon and Bren Comprehensive Cancer Center, Brown Center for Immunotherapy and Vera Bradley Foundation for Breast Cancer, Indiana, IN 46202, USA

Abstract

Cancer immunotherapy with checkpoint inhibitors (ICI) targeting PD-1/PD-L1 axis and CTLA-4 have emerged as a ground-breaking cancer treatment modality for cancer patients exhibiting great clinical outcomes in multiple type of cancers. However, treatment-related side-effects, partial patients' responses and treatment resistance issues are urgently needed to be resolved. In the current study, we examined the efficacy of combination immunotherapy of MVF-TIGIT-2/4 with PD1-Vaxx, PDL1-Vaxx and CTLA4-Vaxx. The results suggest that the combo vaccination of TIGIT plus PDL1-Vaxx significant delayed tumor growth and extended mice survive even better than monoclonal antibody combinations (1G9+10F.9G2). Notably, in D2F2 BALB/c mammary tumor model, the MVF-TIGIT-2 plus PDL1-Vaxx dramatically inhibited tumor growth and showed 100% survive in a certain period which is the best of all the other treatments. The combination immunotherapy including TIGIT-2/4 with PD1-Vaxx and CTLA-4-Vaxx were also examined in syngeneic mouse models with relatively good efficacy. We will present a detailed analysis of the combination immunotherapies and identify the best candidate/s to move into clinical trials.

Translational/Clinical Research ***Faculty***

POSTER #105

CMYC-MEDIATED METABOLIC REPROGRAMMING DRIVEN BY PIM2 INHIBITOR JP11646 PROMOTES CYTOTOXICITY IN TRIPLE-NEGATIVE BREAST CANCER.

Alo RAY[1], Madison Reddock[1], Mateusz Opyrchal[1]

[1] *Department of Internal Medicine, Indiana University School of Medicine, Indiana University Melvin and Bren Simon Cancer Center*

Email: *aloray@iu.edu*

Background: Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer with poor prognosis and lack targeted therapies. Developing new targeted therapies for TNBC is critically important. Pim2, the pro-survival kinase, is overexpressed in breast cancer and other solid tumors compared to normal tissues. We have previously demonstrated that the PIM2 inhibitor, JP11646, showed enhanced cytotoxicity in TNBC cell lines and xenograft mouse models compared to the PIM2 kinase inhibitor AZD1208 supporting a kinase-independent function of JP11646.

Materials and Methods: Breast cancer cell lines (MDA-MB-231, BT549, and MDA-MB-436) were treated with JP11646 or AZD1208 and Western blots and Real-Time PCR were done. Cells were treated with cycloheximide, and protein turnover rate was examined by Western blot. Total mRNA was isolated upon JP11646 and AZD1208 treatments, and mRNA sequencing was done. mRNA sequencing data was analyzed by KEGG pathway analysis and upregulated and downregulated pathways/genes were identified. Key genes were validated by Real-Time PCR.

Results: Our TCGA data analysis showed that PIM2 is overexpressed in basal subtype compared to other breast cancer subtypes suggesting that inhibiting PIM2 might be a novel targeted therapy for TNBC. Overexpression of kinase-dead PIM2 (KD) shows a pro-survival effect suggesting that PIM2 has a pro-survival effect independent of its kinase activity. The KD mutant protein overexpressed cells were more resistant to JP11646 treatment to a similar degree to wild type PIM2 (WT) protein overexpressed cells.Our global mRNA sequencing of JP11646-and AZD1208-treated cells showed that several pathways related to mitochondrial functions and metabolism such as upregulation of oxidative phosphorylation (OXPHOS), ribosome function, cardiac muscle contraction, and downregulation of biosynthesis of nucleotide sugars and aminoacyl tRNA are altered upon JP11646 treatment but not altered upon AZD1208 treatment. Intriguingly, cMYC plays a role in the regulation of several of these pathways altered upon JP11646 treatment. cMYC regulated genes in the OXPHOS pathway important for mitochondrial membrane potential are upregulated upon JP11646 treatment whereas most of the cMYC regulated glycolytic genes were not significantly altered. cMYC is a PIM2 kinase substrate, and therefore, we examined cMYC phosphorylation and total protein levels upon JP11646 and AZD1208 treatments. Both JP11646 and AZD1208 showed a reduction in cMYC phosphorylation. Unexpectedly, JP11646 increased total cMYC protein level in a time-dependent manner. However, AZD1208 did not show this cMYC upregulation supporting that alteration in the cMYC total protein level may be regulated by PIM2 kinase-independent manner. To explore if cMYC degradation is influenced by JP11646, we examined cMYC turnover rate upon JP11646 treatment.When the cells were treated with JP11646 for 24 h followed by cycloheximide, JP11646 treatment reduced PIM2 level faster than vehicle-treated cells. cMYC level increased significantly upon JP11646 treatment and cMYC degradation was slower. JP11646 treatment increased cMYC mRNA level suggesting cMYC transcription is upregulated leading to increase in cMYC protein level.

Conclusion: JP11646 induces metabolic reprogramming by changing the balance between glycolysis and oxidative phosphorylation through a PIM2-cMYC axis. We propose that cMYC deregulation upon JP11646 treatment influences the metabolic reprogramming of glycolytic cancer cells towards OXPHOS, reduces biosynthesis of nucleic acids, and aminoacyl t-RNA promoting cytotoxicity.

Translational/Clinical Research ***Faculty***

POSTER #106

CLINICAL VALIDATION OF A NOVEL COMPREHENSIVE NEXT GENERATION SEQUENCING ASSAY FOR HEMATOLYMPHOID MALIGNANCIES

Lin Wang[5], Lanie Happ[1], Andrea Vrydaghs[2], Jen Loy-Flynn[2], Matt Sperling[2], Kemin Xu[2], Kenneth Ofori[3], Sandeep [4], Magdalena Czader[2]

[1] Data Driven Bioscience, Durham, NC
[2] Department of Pathology and Laboratory Medicine, IUSM, Indianapolis, IN
[3] Department of Pathology and Laboratory Medicine, Indianapolis, IN
[4] Duke University, Durham, NC, Durham, NC
[5] Department of Pathology and Laboratory Medicine, IUSM

Email: *wanglin@iu.edu*

Molecular genetic analysis is integral to diagnosis and targeted therapy of hematolymphoid malignancies with the most recent classifications requiring testing for somatic and germline abnormalities. Next generation sequencing (NGS) has become widely accepted since it provides comprehensive molecular genetic analysis, Nevertheless, access to NGS testing is still limited to academic institutions and reference laboratories due to its complexity, separate DNA and RNA workflows and cost. We present validation and performance of a novel combined DNA/RNA Duoseq assay with integrated bioinformatic pipeline and <5 days turn-around-time, which expands access to NGS testing.

Duoseq protocol was used for total nucleic acid extraction, fragmentation, tagging and library preparation with hybridized capture of 478 genes. Samples were sequenced on Illumina Nextseq 550. Bioinformatic analysis was performed using Duoseq Suite software. Limit of detection (LOD) was confirmed as 5% variant allele frequency (VAF) for SNVs and 10% VAF for indels. Structural variants (SVs) were detectable at ≥20% tumor purity. We studied 121 samples (100 FFPE and 21 fresh) including 60 AMLs, 40 other myeloid neoplasms, and 21 ALLs and mature lymphoproliferative disorders. FoundationOne assay was used for orthogonal comparison. Cell lines (KG1, SUDHL, SUDHL6 and NB4) and contrived samples were also included.

Analytical validation including cell lines and contrived samples demonstrated 100% precision and reproducibility for each variant class for events at or above the LOD. Analytical sensitivity was 98.4% for SNVs, 100% for SVs and 100% for indels for ≥LOD. The specificity was 100%, indicating no instances of cross-contamination of samples within or across runs. We assessed 780 SNVs and achieved a positive predictive value (PPV) of 97.6% and a positive percentage agreement (PPA) of 97.1%. For 120 indels and SSVs examined, the PPV and PPA were 98.4% and 96%, respectively. VAFs measured by Duoseq closely matched those provided by the orthogonal assay (R^2 0.88, fresh samples). We also included 32 cases with common recurrent SV and obtained PPV of 96.8%, PPA of 96.8%, specificity of 99.6% and accuracy of 99.2%. Lastly, the FLT3-ITDs were accessed in 117 cases with PPV 94.7%, PPA 90%, specificity 99% and accuracy of 97.4%.

We determined that Duoseq assay is a reliable diagnostic tool and provides a cost effective and streamlined workflow to test a wide variety of hematolymphoid malignancies.

Translational/Clinical Research ***Faculty***

POSTER #107

THE CHIMERIC ANTIGEN RECEPTOR REDIRECTED MEMORY T CELLS SPECIFIC FOR CD45 ANTIGENS FOR HEMATOLOGIC MALIGNANCIES

Li Zhang[1,3], Riya Sharma[2], Huda Salman[2]

[1] *Department of Microbiology and Immunology, Indianapolis, IN*
[2] *Brown Center for Immunotherapy. IU Simon Comprehensive Cancer Center, School of Medicine, Indianapolis, IN*
[3] *Brown Center for Immunotherapy. IU Simon Comprehensive Cancer Center, School of Medicine*

Email: *lzh6@iu.edu*

Mentor: Huda Salman. hsalman@iu.edu

Background: The primary objective of this project is to engineer chimeric antigen receptor (CAR) T cells to target CD45. CD45 is a transmembrane protein uniformly expressed on hematopoietic cells and progenitors and is also highly expressed in various hematologic malignancies, including leukemias and lymphomas. This ubiquitous expression makes CD45 a compelling therapeutic target. However, its presence on normal hematopoietic cells poses a significant challenge due to on-target, off-tumor toxicity and importantly fratricide during the CAR manufacturing. To metigate those prohebitive limitations, we aim to engineer the CAR in memory T cell (TEM, defined by CD45RA, B & C $^{-}$/ RO^{+}) CD45 antigens, starting with the dominant CD45RA isoform, to eliminate antigen bearing hematologic malignancies as well as a strategy to meyeloablate the marrow in conditioning to stem cell transplant.

Methods: Firstly, we built a third-generation CAR construct expressing a CD45RA-specific single-chain variable fragment (scFv), CD28, and 4-1BB costimulatory domains, produced lentivirus particles, transduced human primary CD45RA-RO+ T cells, and generated the CD45RA-CAR-TEMs. Secondly, we sorted CD45RA^{-}RO^{+} memory T cells (TEMs) and evaluated their memory T cell phenotype during cell expansion when supplied with different dosed of IL-2, IL-7 and IL-15. Thirdly, we harvested CD45RA-CAR-TEMs and tested their antigen specificities and cytotoxic effectiveness on the intended CD45RA-bearing cancer cell lines.

Results: The antigen specificity of the CD45RA-CARs was validated by antigen-dependent secretion of proinflammatory cytokines. The CD45RA-CAR-TEMs recognize targets expressing CD45 isoforms containing CD45RA but not CD45RO, thus mitigated fratricide. CD45RA-RO+ T cells retain their memory T cell feature, expressing CD45RA-RO+CD62L+CCR7+, and expand when supplied with IL-7, IL-15, and low-dose IL-2. In a co-culture assay, the CD45RA-CAR-TEMs kill CD45RA positive Raji cells, CD45RAB positive KG-1 cells and CD45RABC positive HL60 cells, determined by flow cytometry analysis and LDH assay. We also constructed CARs that target the other two isoforms of CD45, CD45RB & CD45RC in testing.

Conclusions: By selectively incorporating the CAR transgene into the CD45RA^{-}RO^{+} TEMs as a proof of principle, we showed the engineered T cells to target alternative CD45 RA containing isoforms—either individually or combined- and such CAR-TEMs exert cytotoxic effect on the antigen-expressing cancer cells. Next, we will evaluate the potent cytotoxic activity of the CD45RA-CAR-TEMs *in vivo* using xenograft models of acute myeloid leukemia and other hematologic malignancies. If successful, this work will serve as the foundation for a clinical trial aimed at eradicating minimal residual disease and establishing an effective pre-transplant conditioning regimen for patients with acute leukemias and lymphomas.

Translational/Clinical Research ***Faculty***

POSTER #108

THE IMPACT OF OXYGEN EXPOSURE ON CLINICAL BIOMARKERS – AN UNDERRECOGNIZED SOURCE OF PRE-ANALYTIC VARIABILITY

Ruizhong Wang[1], Adedeji K. Adebayo[1], Steven Westphal[1], Hala Fatima [1], Matt Thomas[2], Amber Roberts[3], Carla Fisher[1], Mohammad Al-Haddad[1], Sujani Yadlapati[1], Pam Rockey[3], William Berry[1], Emily Nelson[3], April Giron[3], Kathy Miller[1,2,4], Harikrishna Nakshatri[1,3,5]

[1] *Indiana University School of Medicine*
[2] *IU Health*
[3] *IU Melvin and Bren Simon Comprehensive Cancer Center*
[4] *IU Melvin and Bren Simon Comprehensive Cancer Center*
[5] *Richard L Roudebush VA Medical Center*

Email: *rewang@iu.edu*

When preclinical research fails to replicate human biology, scientific progress stalls, clinical trials falter, and patients continue to suffer. While many factors contribute to these failures, lack of attention to pre-analytic variability is a seminal issue. We recently reported that even short-term exposure to ambient air is sufficient to trigger signaling changes in tumor and non-malignant biospecimens. Those changes in turn alter their biology and responsiveness to targeted therapies. Thus, characterization of tumors collected and processed under physioxia (3% O_2) instead of current practice of collection and processing under ambient air (21% O_2) will help to identify clinically relevant biomarkers that are affected by O_2 tensions. This approach may help to reduce clinical trial failure rates and increase clinical translation of preclinical studies. Towards this goal, we collected human specimens (biopsies, ascites and pleural effusions) under physioxia, then divided the same specimen into two groups; one group maintained under physioxia for 45-60 minutes before fixing/processing and the other group exposed to ambient air for 45-60 minutes before fixing/processing. Samples were subjected to various biomarker analysis using immunohistochemistry, Western blotting, and qRT-PCR. Tumor cells cultured for few days under physioxia and ambient air and sorted for $EpCAM^+$ epithelial (potentially enriched for tumor cells) and non-epithelial cells were subjected to nanopore sequencing to determine O_2 tension-dependent changes in DNA methylation. We found the levels of p-ERK and p-AKT, two clinically used biomarkers of targeted therapy response, are significantly higher in esophageal cancerbiopsies and lobular carcinoma of breast cancer biopsies under physioxia compared to those biopsies exposed to ambient air. Similar effects of O_2 tension on p-ERK and p-AKT levels were observed in cells isolated from ascites or pleural effusion. The effects of O_2 tension on biomarkers showed specificity as we did not observe significant differences in pEGFR under two conditions. The observed differences in signaling pathways extended to cultured cells from ascites fluids and pleural effusions. For example, phosphorylated *p*53 was increased in cultured $EpCAM^+$ epithelial cells under ambient air compared to cells under physioxia suggesting that exposure of tumor specimens to ambient air activates p53 pathways. Indirectly, these results suggest that *p*53 mutation status determines the effects of O_2 tension on levels of specific biomarkers in tumor specimens. O_2 tension-dependent differences in DNA methylation of specific CpG islands on chromosome 6, 10, 13, and 22 in $EpCAM^+$ cells and chromosome 17 in $EpCAM^-$ cells were also observed. Collectively, our current study lays out a new physiologically relevant biomarker validation/discovery platform, which may accelerate evaluation of physiologically relevant signaling networks, new drug discovery, and enhance clinical translation of preclinical observations.

Translational/Clinical Research ***Faculty***

POSTER #109

GERMLINE LOSS-OF-FUNCTION REGULATORY VARIANT OF ACKR1 GENE AND ITS INFLUENCE ON NORMAL BREAST AND BREAST CANCER BIOLOGY

Stephanie Adama[3], Adedeji Adebayo[1], Sedat Kacar[2], Poornima Bhat-Nakshatri[2], Jiang Guanglong[2], Cihat Erdogan[2], Bryan Schneider[2], Kathy D. Miller[2], Harikrishna Nakshatri[2]

[1] *Emory University/Winship Cancer Institute, Atlanta, GA*
[2] *Indiana University School of Medicine, Indianapolis, IN*
[3] *Indiana University School of Medicine*

Email: *sadama@iu.edu*

The *Atypical Chemokine Receptor 1*(*ACKR1/DARC*) gene plays a major role in regulating immune/inflammatory pathways by functioning as a decoy receptor for several cytokines/chemokines including the breast cancer metastasis-associated CXCL12. *ACKR1* gene harbors several single nucleotide variants in the regulatory and coding regions. The regulatory region variant *rs2814778* is responsible for the Duffy-Null (*CC*) /heterozygous (*TC*) phenotype, which results in significant reduction in ACKR1 expression in both epithelial and non-epithelial cells. The *rs2814778* variant is enriched in African and Arab ancestry. ACKR1 germline variants are embedded in human population to protect against malarial infection but altered chemokine/cytokine signaling in individuals with these variants influence cancer progression pathways. It has recently been suggested that clinical trial design need to take Duffy phenotype/genotype into consideration to account for normal biological differences. We investigated the potential influence of *ACKR1* variant in breast cancer using three approaches. The first approach was analysis of the E5103 breast cancer clinical trial dataset, which showed enrichment of the *ACKR1 rs2814778* variant correlating with lower Disease-free survival (DFS) rate (HR = 1.5, p=0.0014 for TC/CC vs TT). The second approach was analysis of the UALCAN database, which showed lower expression of ACKR1 in breast cancer correlating with progression to brain metastases. The third approach was the establishment of a model system to determine the influence of rs*2814778* variants on breast progression with a long-term goal of identifying therapeutic vulnerabilities. We utilized breast tissues from the institutional resource of Komen Normal Tissue bank and generated immortalized breast epithelial cell lines with functional *TT* (wild type that express ACKR1 -African and European ancestry), heterozygous (*T/C*) and homozygous (*CC*). Cell lines with *TT* expressed higher levels of ACKR1 mRNA compared to those with *T/C* or *CC* in the regulatory region. We assessed the basal activity of receptor tyrosine kinases, which may contribute to cell-autonomous mechanisms in inflammation-driven cancer, and secreted chemokines/cytokines in these cell lines. We observed African ancestry and/or ACKR1 expression-dependent variations in phosphorylation of Hepatocyte Growth Factor Receptor (HGFR/c-Met), Insulin -like Growth Factor Receptor-1 (IGFR-1) and Fibroblast Growth Factor Receptor 3 (FGFR3) in these cell lines. Since loss/reduction of ACKR1 alters chemokine homeostasis, we sought to explore further downstream oncogenic pathways which may be activated by autocrine or paracrine mechanisms. We observed that ACKR1 appears to influence WNT/GSK3β signaling by differentially phosphorylating β-catenin. *In vivo* studies are underway to determine whether ACKR1 expression levels in transformed cells correlate with distinct tumor characteristics including cancer stem cell properties, metastasis propensity and response to targeted and conventional chemotherapies. Comprehensive analyses of *ACKR1* germline variants may provide additional insights into whether these variants need to be taken into consideration for clinical decision-making including treatment options.

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

POSTER #110

INVESTIGATING THE THERAPEUTIC POTENTIAL OF A NOVEL PRMT5 INHIBITOR FORMULATED WITH NANOCRYSTAL TECHNOLOGY IN PANCREATIC DUCTAL ADENOCARCINOMA

Faranak Alipourgivi[1,2], Zhongyue (Claire) Yuan[3], Rahaf Habboub[4], Yoon Yeo[3], Tao Lu[4,5,6,7]

[1] *Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN USA*
[2] *Experimental and Developmental Therapeutics Program, Indiana University Melvin and Bren Simon Comprehensive Cancer Center*
[3] *Department of Industrial and Molecular Pharmaceutics, Purdue University, West Lafayette, IN, USA*
[4] *Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN, USA*
[5] *Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN, USA*
[6] *Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA*
[7] *Experimental and Developmental Therapeutics Program, Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN, USA*

Email: *falipour@iu.edu*

Persistent inflammation is a defining feature of pancreatic ductal adenocarcinoma (PDAC), largely driven by sustained activation of the NF-κB signaling pathway. Disruption of NF-κB regulatory mechanisms contributes significantly to this chronic activation. Protein arginine methyltransferase 5 (PRMT5), a known promoter of tumorigenesis in several cancers—including PDAC, colorectal, and breast—has emerged as a promising therapeutic target. Clinical data reveal that PDAC patients with high PRMT5 expression have significantly shorter median survival, underscoring its prognostic and therapeutic relevance. Our lab has been developing small-molecule inhibitors targeting PRMT5. Among these, our patented compound, PR5-LL-CM01 (CM01), has shown superior anti-tumor efficacy and reduced toxicity in PDAC models compared to the commercially available PRMT5 inhibitor EPZ015666. However, CM01's poor water solubility poses a challenge for clinical translation. To address this, we aim to enhance its solubility and bioavailability by formulating CM01 as an albumin-coated nanocrystal (NC) (Abxtal). Preliminary studies confirmed successful production and physicochemical characterization of CM01 NCs, with an optimized particle size (Z-average ~87 nm), and storage stability maintained for at least three months. In this project, we assess the therapeutic potential of CM01 NC in vitro and in vivo. We hypothesize that CM01 NC more effectively inhibits PRMT5-mediated NF-κB signaling and associated oncogenic processes than unformulated CM01, and that it synergizes with gemcitabine (Gem) to suppress PDAC progression. Supporting this hypothesis, CM01 NC showed equal or greater inhibition of PDAC PANC1 and MIA PaCa2 cell growth compared to CM01, and more effectively suppressed 3D spheroid growth and cell migration in vitro. CM01 NC also outperformed CM01 in reducing NF-κB transcriptional activity, with comparable reductions in NF-κB target genes (TNF-α and IL-8) per qPCR assays. Additionally, Chou-Talalay analysis demonstrated that CM01 NC exhibits synergistic activity with Gem. We are now transitioning to in vivo studies to evaluate the pharmacokinetics and therapeutic efficacy of CM01 NC, both as a monotherapy and in combination with Gem, using PDAC models. This study may establish CM01 NC, alone or with Gem, as a promising therapeutic strategy, and could lay the foundation for clinical development of CM01 NC-based treatments for PDAC.

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

POSTER #111

PREDICTIVE MODELING OF DIFFERENTIAL TARGETING AND ADDITIVE EFFECTS OF CDK4/6 INHIBITORS IN MPNST

Colin Beach[1,6], Christopher Davis[2], Shelley Dixon[2], Sarah Morrow[3], Christine Berryhill[2], Steven Rhodes[4], D. Wade Clapp[4], Steven Angus[5]

[1] *Melvin and Bren Simon Comprehensive Cancer Center, Indiana University School of Medicine, Indianapolis, IN*
[2] *Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN*
[3] *Department of Pediatrics, Indiana University School of Medicine, Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN*
[4] *Department of Pediatrics, Indiana University School of Medicine, Melvin and Bren Simon Comprehensive Cancer Center, Indiana University School of Medicine, Indianapolis, IN*
[5] *Department of Pediatrics, Indiana University School of Medicine, Melvin and Bren Simon Comprehensive Cancer Center, Indiana University School of Medicine, Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN*
[6] *Department of Pediatrics, Indiana University School of Medicine*

Email: *cobeach@iu.edu*

Purpose: The leading cause of premature death for patients with Neurofibromatosis type 1 (NF1) is the development of malignant peripheral nerve sheath tumors (MPNST). These tumors render current medical treatment strategies largely ineffective, prompting an investigation of rational targeted therapies. CDK4/6 inhibitors are favorable candidates to oppose the chronic cell cycle deregulation in NF1-related MPNST, but continued characterization of tumor adaptive responses and the polypharmacology of these drugs is needed.

Methods: Multiplexed kinase inhibitor bead (MIB) affinity chromatography coupled with mass spectrometry (MIB/MS) was used to identify target spectra of CDK4/6 inhibitors abemaciclib and palbociclib. *In vitro* changes in kinase activity and expression were also analyzed by MIB/MS following treatment of MPNST cell lines. CRISPR/Cas9 knockout and shRNA knockdown of *RB1* in MPNST cell lines were used to evaluate the dependence of CDK4/6 inhibitors on RB.

Results: MPNST cell lines demonstrated sensitivity to single agent treatment by CDK4/6 inhibition, inclusive of reduced cell viability and cell cycle entry. In MIB/MS competition assays, previously reported off-target kinases exclusive to abemaciclib were identified, while palbociclib remained selective to CDK4/6. Both inhibitors elicited diverse kinome response profiles despite a shared cell cycle arrest phenotype. Inhibition of unique secondary (non-CDK4/6) targets of abemaciclib demonstrated greater additive effects in combination with abemaciclib than with palbociclib. *RB1* knockout and knockdown MPNST cell lines exhibited resistance to palbociclib treatment, but remained sensitive to abemaciclib, in long-term exposure studies.

Conclusions: CDK4/6 inhibition continues to show promise as a targeted therapy against NF1-related MPNST with dysregulated cell cycle signaling. The discovery of additive combination therapies based on identified non-CDK4/6 kinase targets highlights the translational capacity of predictive kinome profiling. Further characterization of abemaciclib combination therapies and their *in vivo* efficacy is therefore warranted.

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

POSTER #112

SEEING THE WHOLE PICTURE: DEEP LEARNING-BASED TISSUE SEGMENTATION: IMPROVING THE DETECTION OF PROSTATE CANCER USING WHOLE-MOUNT HISTOPATHOLOGY FROM RADICAL PROSTATECTOMY SPECIMENS

Yamlak Bogale[2], Katrina Collins[3], Clinton Bahler[1,4], Michael Feldman[3], Rakesh Shiradkar[2]

[1] *Department of Radiology and Imaging Sciences, Indiana School of Medicine, Indianapolis, IN, USA , Indianapolis, IN*
[2] *Department of Informatics, Luddy School of Informatics, Computing, and Engineering, Indianapolis, IN, USA*
[3] *Department of Pathology and Laboratory Medicine, Indiana School of Medicine, Indianapolis, IN, USA*
[4] *Department of Urology, Indiana School of Medicine, Indianapolis, IN, USA*

Email: *ybogale@iu.edu*

Background: Prostate cancer (PCa) is one of the most commonly diagnosed cancers in men. The Gleason grading system assesses PCa aggressiveness by evaluating cancer cell patterns. Tumor-adjacent regions, particularly the stroma, provide important prognostic information. The stroma, supportive tissue surrounding the tumor, influences cancer progression due to changes in cellular composition and extracellular matrix remodeling. Alterations in the stroma are associated with biochemical recurrence and poorer prognosis in PCa patients. Pathologists traditionally annotate tumor regions in histopathology images, a time-consuming process due to the high resolution of these images. This challenge is amplified with whole-mount histopathology, where the entire prostate gland is sectioned and placed on oversized slides, offering a full cross-section of the prostate. Deep learning has enabled automatic segmentation in digital pathology, allowing for efficient analysis of large, high-resolution images.

Objective: The goal of this project is to evaluate pre-trained tissue segmentation models to discover biomarkers within tumor-adjacent stroma regions, identifying potential markers for PCa prognosis and treatment.

Methods: To overcome manual annotation challenges, we aimed to automatically generate segmentation masks for various prostate tissue regions using a deep learning model. This approach improves the speed and accuracy of PCa diagnosis. The model was pre-trained using the Automated Gleason Grading Challenge 2022 (AGGC22) dataset, which includes biopsy and whole-mount histopathologic images. The trained model was evaluated on N=48 whole-mount histopathology images from patients who underwent radical prostatectomy at Indiana University School of Medicine. The deep learning model was evaluated on 48 H&E-stained whole-mount histopathology images digitized at 20x magnification. The images were annotated by an experienced genitourinary pathologist (K.C.). The primary objective was to assess the model's ability to classify different tissue regions in whole-mount histopathology images.

Results: The segmentation results demonstrated promising results in segmenting prostate tissue regions, classifying them into stroma (supportive tissue), benign (non-cancerous tissue), and malignant (Gleason patterns 3, 4, and 5). This was particularly evident when comparing the ground truth annotations of the tumor region with the regions segmented as Gleason patterns 3, 4, and 5, where an overlap occurred between the tumor annotations and the segmentation masks of these malignant regions.

Conclusion and Discussion: This deep learning model demonstrated promising results in automated segmentation of prostate tissue regions, reducing the need for manual annotations and improving processing times. Despite being trained on images with varying structures and sizes, the model adapted well to a completely different dataset, showcasing its generalization capability. This study serves as a proof of concept for leveraging automated segmentation to enhance the speed and accuracy of PCa diagnosis. With further refinement, it can be adapted to better study tumor-adjacent stromal morphology.

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

POSTER #113

DIFFERENTIAL GENE EXPRESSION REGULATION BY THE RXR AGONIST V-125 IN MMTV-NEU MAMMARY TUMORS

Afrin Sultana Chowdhury[5], Lyndsey A. Reich[1], Christopher J. Occhiuto[1], Elizabeth Yeh[2], Ana Leal[3], Karen Liby[4]

[1] *Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824, USA , East Lansing, MI*
[2] *Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46202, USA, Indianapolis, IN*
[3] *Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202, USA*
[4] *Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202, USA, Indianapolis, IN*
[5] *Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46202, USA*

Email: *afschow@iu.edu*

Retinoid X receptors (RXRs) are nuclear receptors that play a pivotal role in regulating gene expression by forming homodimer and heterodimers with other nuclear receptors. When activated, RXRs influence various cellular processes, including cell differentiation, proliferation, and apoptosis. This regulatory activity makes RXR agonists promising candidates in cancer research. However, the specific gene expression changes induced by RXR agonists can vary depending on the compound, reflecting their unique interactions within cellular environments and signaling networks. In this study, RNA sequencing was performed to compare the molecular pathways activated by the novel compound V-125 and FDA-approved drug bexarotene in mammary tumors of a mouse model of HER2+ breast cancer (MMTV-Neu mice). By analyzing the transcriptomic profiles, we identified that each treatment differentially regulated cancer-relevant gene categories, including focal adhesion, regulation of actin cytoskeleton, and extracellular matrix-receptor interaction. The most significantly altered genes by V-125, including *Lrrc26, Mgarp, Aldh1a3, and Colca2* are positively correlated with improved survival in breast cancer patients. While V-125 and bexarotene act on many common pathways, they induce distinct gene expression profiles. V-125 primarily targets focal adhesion and extracellular matrix-receptor interaction pathways, whereas bexarotene affects regulation of actin cytoskeleton and NF-kappa B signaling pathways. We confirmed the significant differential expression ($p<0.05$) of key genes (*Nrg1, Nfasc, Chi3l1, Slit2*) at both the mRNA and protein levels using quantitative PCR (qPCR) and immunohistochemistry (IHC). Beyond cancer cells, RXR modulation influences the tumor microenvironment, including immune cells. Our transcriptomic analysis revealed that RXR agonist treatment affects pathways associated with macrophage activation and phagocytosis. This suggests the role of RXR in polarization of macrophage, an important immune population related to breast cancer. To investigate this further, we utilized bone marrow-derived macrophages (BMDMs) to evaluate the impact of V-125 and bexarotene treatment on macrophage polarization toward a tumor-suppressive phenotype. The polarization status of macrophages upon treatment was assessed through gene expression analysis by qPCR. We observed that BMDMs treated with 300nM of either RXR agonist significantly ($p <0.05$) increased mRNA expression of *IRF7* and *TLR9*, associated with a pro-inflammatory, anti-tumor phenotype. This comprehensive approach allowed us to determine the relative effectiveness of V-125 and bexarotene in modulating gene expression and polarizing macrophages toward a tumor-suppressive phenotype, providing valuable insights into their therapeutic potential and the underlying molecular mechanisms.

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

POSTER #114

EXPLORING THE CELLULAR EFFECTS OF DIS3 HOTSPOT AND NON-HOTSPOT MUTATIONS IN MULTIPLE MYELOMA

Ceanne Elliott[1,2], Enze Liu[2], Parvathi Sudha[2], Nathan Becker[1,2], Charlotte Pawlyn[3], Amber Mosley[2,4], Brian Walker[2,5]

[1] *Department of Medical and Molecular Genetics, Indiana University School of Medicine*
[2] *Simon Comprehensive Cancer Center, Indiana University School of Medicine*
[3] *Division of Cancer Therapeutics, Institute of Cancer Research*
[4] *Center for Proteome Analysis, Indiana University School of Medicine*
[5] *Division of Myeloma, Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine*

Email: *ceelli@iu.edu*

Multiple myeloma (MM), the second most common hematological cancer, is associated with a poor prognosis which varies based on the genetic alterations present at the time of diagnosis. Missense mutations in DIS3 are found in 10% of MM patients and can be either recurrent heterozygous hotspot mutations or homozygous non-hotspot mutations, which are associated with a worse prognosis. However, the differential impact of these mutation groups on cellular functions has yet to be investigated. This knowledge is crucial to understanding the biological role DIS3 mutations play in the progression of MM. To determine the function of these mutation groups, we analyzed the Multiple Myeloma Research Foundation CoMMpass Study of patient samples (n=1,245) which underwent DNA and RNA sequencing. We complemented this analysis by utilizing CRISPR-Cas9 homology directed repair to introduce 7 mutations (3 hotspot; 4 non-hotspot) into endogenous DIS3 in the MM cell line KMS11, which were analyzed by multi-omics. The introduction of DIS3 mutations did not affect DIS3 mRNA levels, although non-hotspot mutations resulted in a 25-fold decrease ($p<0.0001$) in DIS3 protein expression. In contrast, hotspot mutations exhibited no protein expression change, as confirmed by mass spectrometry. Non-hotspot DIS3-mutated protein loss was also observed in a Western blot of MM patient bone marrow samples, therefore confirming the translational impact of our cell line results. Loss of DIS3 expression in the non-hotspot mutations was not rescued by proteasome inhibition, implicating that the mechanism of mutated-DIS3 protein loss is not accounted for by proteasome degradation. Total RNA-sequencing and differential gene expression analysis revealed DIS3 mutations result in a large increase ($p<0.0001$) of lncRNAs, with non-hotspot mutations having significantly more of an increase ($p=0.0032$) than hotspot mutations. These results were also observed in the CoMMpass Study dataset, supporting the validity of our DIS3-mutated cell lines. Over half of the differentially expressed genes resulting from both hotspot and non-hotspot mutations were found to be regulated by AU-rich elements, suggesting aberrant post-transcriptional regulation which may contribute to disease pathology. Gene set enrichment analysis also showed non-hotspot mutations led to a greater downregulation of genes involved in RNA processing, transcription, and protein processing pathways compared to hotspot mutations. Analysis of mass spectrometry data indicated that non-hotspot mutations greatly impact the proteome by resulting in the significant ($p<0.05$) abundance change in approximately 1,000 proteins, whereas hotspot mutations resulted in the abundance change of 15 proteins. In addition, only non-hotspot mutations resulted in the significant ($p<0.05$) dysregulation of almost half of the subunits in the RNA exosome on the protein level. Our findings provide insight into the cellular impact of DIS3 mutations, highlighting key differences between mutation groups which aid in furthering our understanding of MM pathogenesis.

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

POSTER #115

A JAGGED1-NOTCH3 MEDIATED TUMOR-STROMAL JUXTACRINE CROSSTALK ACTIVATES A SUBPOPULATION OF αSMA+ECM REMODELING MYOFIBROBLASTIC CAFS IN HGSOC METASTASIS

Argha Ghosh[1,2], Ji Wang[1,2], Xue Xiao[1,2], Anirban K Mitra[2,3]

[1] *Indiana University School of Medicine, Bloomington, IN*
[2] *IU Simon Comprehensive Cancer Center*
[3] *Medical and Molecular Genetics, Indiana University School of Medicine, Bloomington, IN*

Email: *arghosh@iu.edu*

Ovarian cancer (OC) is the most lethal gynecological malignancy, with high-grade serous ovarian cancer (HGSOC) as its deadliest subtype due to widespread metastasis. Metastatic mechanisms, responsible for ~90% of cancer-related deaths, remain elusive and challenging to target. Cancer-associated fibroblasts (CAFs), comprising 10–50% of the metastatic tumor microenvironment (TME), drive key metastatic processes, including extracellular matrix (ECM) remodeling, angiogenesis, and immune modulation. However, critical gaps persist in understanding how distinct CAF subtypes emerge and contribute to metastasis. CAFs exhibit significant heterogeneity, with key subtypes including myofibroblastic CAFs (myCAFs), which remodel the ECM, and inflammatory CAFs (iCAFs), which modulate the immune response. Deciphering the mechanisms governing CAF activation and heterogeneity is crucial for targeting their pro-metastatic functions. While tumor-driven paracrine signaling is known to activate CAFs, the role of tumor-stromal juxtacrine interactions in this process remains largely unexplored. Using a combination of bioinformatics and in vitro approaches, we uncovered critical juxtacrine signaling dynamics between metastasizing OC cells and CAFs, shedding new light on CAF activation and heterogeneity. By analysing the molecular subtypes of HGSOC in TCGA, in conjunction with scRNA-seq data from 11 patient metastases, we identified 9 distinct CAF subpopulations exhibiting reciprocal myCAF/iCAF signatures. Ligand-receptor analysis using CellChat revealed a pivotal OC-CAF communication axis, where Jagged1 ligand-expressing OC cells engage Notch3 receptor in CAFs. Notably, high Notch3 expression in patient CAFs was strongly associated with the myCAF subtype. A second scRNA-seq of a 3D heterotypic coculture model using ascites-derived primary HGSOC cells and metastasis-isolated CAFs, successfully recapitulated the OC-CAF crosstalk. Immunofluorescence staining of OC-CAF cocultures confirmed the spatial induction of Notch3 and the myCAF marker alpha smooth muscle actin (αSMA) in CAFs juxtacrine to Jagged1-expressing OC cells. Genetic knockdown studies further reaffirmed Notch3 as the central regulator of myofibroblastic CAFs, with Notch3 loss reducing Hes1 and αSMA expression in three independent patient-isolated CAFs. This reduction was accompanied by decreased collagen contraction, a hallmark myCAF function. Furthermore, Jagged1 emerged as the critical ligand for driving the specific activation of Notch3. Interestingly, bulk RNA-seq using two Notch3 activation approaches—NICD3 overexpression and Jagged1 stimulation—identified a unique 272-gene transcriptome specific to the Jag1-Notch3 axis. The top 20 transcriptional pathways confirmed activation of myCAF functionalities, including ECM organization, cell-cell adhesion, contractility, and angiogenesis through the Jag1-Notch3 axis. Notably, 130 of these genes also exhibited increased enhancer chromatin accessibility in NICD3 overexpressing CAFs as potential mechanism of regulation, revealed by ATAC-seq. Clinically, elevated Notch signaling in CAFs correlated with poor prognosis in HGSOC patients and treatment with LY411575, a γ-secretase inhibitor reduced collagen contraction, indicating therapeutic potential. Therefore, in the long term, targeting this axis can potentially translate into effective therapeutics for TME "normalization" for metastatic HGSOC.

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

POSTER #116

Remove

IMPLEMENTATION OF EVIDENCE-BASED SYMPTOM MANAGEMENT ALGORITHMS TO REDUCE THE EMERGENCY ROOM ENCOUNTERS

Ashley Hums[4], Kimberley Bernstein[1], Lisa Greenan[2], Stacey Ross[2], Susan Storey[3]

[1] *IU Health Simon Cancer Center , Indianapolis, IN*
[2] *IU Simon Cancer Center , Indianapolis, IN*
[3] *Indiana University School of Nursing , Greenwood , IN*
[4] *Indiana University School of Nursing*

Email: *ashschaf@iu.edu*

Background & Significance

Oncology patients often seek emergency room (ER) care for symptom management. Accessing care through the ER can result in increased risks for: nosocomial infections, treatment delays, hospitalization/rehospitalization, and healthcare costs.[1, 2] Early identification/intervention for symptom management is crucial for improving patient outcomes. Implementing evidence-based symptom management (EBSM) algorithms may reduce ER encounters.

Purpose

To determine: 1) the most common symptoms reported 2) the EBSM algorithm and category of recommendations used, and 3) if EBSM algorithms decreased ER encounters.

Methods

EBSM algorithms were implemented to address the nine most common symptoms (pain, nausea/vomiting, diarrhea, fatigue, hair/skin/nails, constipation, fever, peripheral neuropathy, and mucositis). The EBSM algorithms used a “stop light method” red (severe), yellow (moderate) green (mild) to categorize symptom severity and guide recommendations.

Data Collection/Analysis

Pre-implementation data included the most common symptoms reported and the number of ER encounters (per month). Post implementation data collection included the previous metrics, plus specific EBSM algorithm and category of recommendation. Descriptive statistics and a one-tailed Independent Samples t test (alpha 0.5) were conducted to examine the difference in ER encounters before/after implementation.

Results

Pre-implementation the most common symptoms reported were nausea, vomiting, and diarrhea, with 286 ER encounters. Post-implementation EBSM algorithms were used 193 times for 214 oncology patients, addressing symptoms such as pain (n= 42), nausea/vomiting (n= 38), diarrhea (n= 28), fatigue (n= 27), hair/skin/nails (n= 23), constipation (n=13), fever (n=9), peripheral neuropathy (n=7), and mucositis (n=6). Recommendations fell into red (n=18), yellow (n=39), and green (n=98) categories. Post-implementation ER encounters decreased by 27% from 286 to 208, indicating a significant difference between pre- and post-implementation groups t (2.24), p=.033), with the pre-group having more ER encounters *(M =72, SD =10)* than the post-group *(M =52, SD 14)*. The effect size between the pre- and post-implementation groups was calculated using Cohen’s d, resulting in a value of 1.64, indicating a large effect.

Discussion

EBSM algorithms can effectively guide clinicians in symptom assessment and management, potentially improving patient outcomes and reducing ER encounters.

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

POSTER #117

UNDERSTANDING THE ROLE OF MSWI/SNF COMPLEX IN NEUROENDOCRINE TRANSDIFFERENTIATION OF PROSTATE CANCER

Sukhman Kaur[1]

[1] *Purdue University, Borch Department of Medicinal Chemistry and Molecular Pharmacology (BMCMP)*

Email: *kaur259@purdue.edu*

Prostate cancer (PCa) ranks as the primary cause of cancer-related mortality in men within the United States. The progression of prostate cancer is influenced by the binding of androgens to the androgen receptor (AR). Consequently, the primary treatment approach is androgen deprivation therapy (ADT), which typically results in initial disease regression.[2] However, prostate cancer often develops resistance to ADT, evolving into a more aggressive form known as "castration-resistant prostate cancer" (CRPC), which further transitions to a treatment emergent neuroendocrine-like prostate cancer (NEPC) through neuroendocrine differentiation (NED). This progression is marked by the loss of conventional androgen receptor signaling and the activation of neuroendocrine pathways. The mammalian SWItch/Sucrose non-fermenting (mSWI/SNF) or BRG1/BRM-associated factor (BAF) complex is a chromatin remodeling complex, which remodels the nucleosomes, make DNA accessible for transcription, and eventually regulate gene transcription. This multi-subunit complex governs the differentiation of neuronal progenitor cells into various cell subtypes, such as neurons, by transitioning from npBAF, which functions in neural stem and progenitor cells, to neuron-specific BAF (nBAF). Therefore, this change in BAF composition may play a role in the transition from CRPC to NEPC, a relationship that has not yet been thoroughly investigated. In this project, we aim to determine the changes in mSWI/SNF or BAF complex subunit composition and the functional consequence of this change during neuroendocrine differentiation (NED) in prostate cancer. The overall goal is to determine novel therapeutic targets for NEPC.

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

POSTER #118

DUAL INHIBITION OF CDK4/6 AND MEK IN ANAPLASTIC PLEOMORPHIC XANTHOASTROCYTOMA PRECLINICAL MODELS: INTERROGATION OF THERAPEUTIC RESPONSE MECHANISMS

Jenna Koenig[1,8], M. Reza Saadatzadeh[2], Barbara J. Bailey[2], Pankita Pandya[2], Erika Dobrota[2], Kathryn Coy[3], Felicia Kennedy[3], Anthony Sinn[3], Jignesh Tailor[4], Steven Angus[5], Nur Damayanti[6], Karen Pollok[7]

[1] *Wells Center for Pediatric Research, Indiana University Simon Comprehensive Cancer Center, Indianapolis, IN*
[2] *Department of Pediatrics, IUSM, Wells Center for Pediatric Research, Indiana University Simon Comprehensive Cancer Center, Indianapolis, IN*
[3] *Indiana University Simon Comprehensive Cancer Center, Indianapolis, IN*
[4] *Department of Neurosurgery, Wells Center for Pediatric Research, Indiana University Simon Comprehensive Cancer Center, Indianapolis, IN*
[5] *Indiana University Simon Comprehensive Cancer Center, Department of Pediatrics, IUSM, Wells Center for Pediatric Research, Indianapolis, IN*
[6] *Department of Neurosurgery, Indianapolis, IN*
[7] *Department of Pediatrics, IUSM, Indiana University Simon Comprehensive Cancer Center, Wells Center for Pediatric Research, Indianapolis, IN*
[8] *Department of Pediatrics, IUSM*

Email: *jkkoenig@iu.edu*

Typical survival for pediatric high-grade gliomas remains less than 18 months despite recent improved understanding of the molecular drivers of these tumors. Hyperactivating MAPK and CDK4/6 pathway mutations are common and targetable alterations implicated in tumorigenesis and malignant transformation in pediatric glioma. We have established and characterized a novel patient-derived xenograft (PDX) model, RHT128, from a pediatric patient diagnosed with the high-grade glioma anaplastic pleomorphic xanthoastrocytoma (APXA). The molecular landscape of PDX RHT128 exhibits molecular fidelity to the patient's tumor. Clinical precision genomics analysis of the tumor revealed a novel BRAF fusion protein and CDKN2A/B deletion. 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. In this study our objective is to simultaneously target the CDK4/6 and MAPK pathways in RHT128 as well as two additional APXA PDX models, D2363 (BRAF wild-type) and D645 (BRAFV600E mutant) that show CDK4/6 hyperactivation. We aim to determine to what extent this combination therapy minimizes emergence of therapeutic resistance. Single-agent efficacy assessments in a subcutaneous RHT128 PDX showed significant dose-dependent reduction in tumor volume after treatment with the blood-brain barrier-permeable abemaciclib ($p<0.05$) and MEK inhibitor mirdametinib ($p<0.0001$). Analysis of the global kinome in CDK4/6 inhibitor-treated PDX tissues compared to vehicle treatment using multiplexed-inhibitor bead chromatography–mass spectrometry demonstrated effective inhibition of CDK4/6 and dose-dependent increases in MAPK pathway kinases, supporting a combined MEK and CDK4/6 inhibitor therapeutic strategy. Indeed, RHT128 PDX showed improved survival after treatment with combined mirdametinib and abemaciclib compared to either monotherapy or vehicle. An improvement in survival was not observed in the BRAF-WT D2363 PDX treated with this combination, suggesting that BRAF status may be a marker of response to this treatment. Examination of the molecular response in these treated tumors is ongoing and will investigate potential mechanisms of therapeutic resistance. Future studies include testing this combination in the D645 model and in intracranial PDX models of RHT-128, D2363, and D645 which will provide important insight into the blood-brain barrier penetrability of the combination and how the brain microenvironment may impact efficacy. The pervasiveness of alterations to the MAPK and CDK4/6 pathways in pediatric gliomas make this approach promising for further study in a broader range of these deadly tumors.

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

POSTER #119

IDENTIFYING SYNTHETIC LETHAL KINASES IN A MYCN-OVEREXPRESSED NEUROEPITHELIAL STEM CELL MODEL OF SHH-TYPE MEDULLOBLASTOMA

Jenna Koenig[1,7], Victoria Dershem[2], Scott Cooper[3], Titto Augustine[4], Steven Angus[5], Jignesh Tailor[6]

[1] *Wells Center for Pediatric Research, Indiana University Simon Comprehensive Cancer Center, Department of Pediatrics, IUSM, Indianapolis, IN*
[2] *Department of Neurosurgery, IUSM, Indianapolis, IN*
[3] *Department of Neurosurgery, Indianapolis, IN*
[4] *Department of Neurosurgery, Indiana University Simon Comprehensive Cancer Center, Indianapolis, IN*
[5] *Department of Pediatrics, IUSM, Wells Center for Pediatric Research, Indiana University Simon Comprehensive Cancer Center, Indianapolis, IN*
[6] *Department of Neurosurgery, Wells Center for Pediatric Research, Indiana University Simon Comprehensive Cancer Center, Indianapolis, IN*
[7] *Department of Neurosurgery, IUSM*

Email: *jkkoenig@iu.edu*

Medulloblastomas (MB) are the most common malignant brain tumor in children and a leading cause of cancer-related death. The Shh-MB subgroup presents the worst prognosis, and additionally is enriched with mutations in the prevalent oncogene MYCN. MYCN is 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 several 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. Here, we aim to leverage a model overexpressing MYCN in neuroepithelial stem cells (MYCN-NES) to interrogate synthetic lethal kinases that may be targeted with a kinase inhibitor for the treatment of Shh-MB. MYCN-NES have been shown to be tumorigenic in mice and reliably form Shh-MB subgroup medulloblastomas. NES cells derived from the embryonic hindbrain were transfected with a plasmid containing hMYCN and a neomycin-resistance gene for selection. After MYCN overexpression and retention of NES characteristics were confirmed, both wild-type and edited cell lines were transfected with Brunello human kinome knockout sgRNA libraries 1-4. Amplicons derived from both cell lines at Day 0 and Day 14 were sequenced to determine which kinases were knocked out in the surviving cells. Hits from our synthetic lethal screen centered on the MAPK, Hippo, Wnt, and cell cycle pathways and will be validated by global activated kinome profiling of iPSC-derived MYCN-NES intracranial tumors to further identify targets for future pharmacologic study. In the current study, we have demonstrated the feasibility of using hindbrain NES cells as an in vitro model for MYCN overexpression, such as that found in Shh-type medulloblastoma, and the ability to perform CRISPR knockout screens in these cells. Furthermore, with this model, we were able to identify potential synthetic lethal kinases in our overexpression model that may provide a method of indirect pharmacological intervention in these cells. Future studies will employ FDA-approved kinase inhibitors of our top hits for testing against our MYCN-NES in vitro, our MYCN-NES intracranial models, as well as further investigation of the global activated kinome in Shh-MB patient tissues.

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

POSTER #120

TARGETING DNA REPAIR PATHWAYS IN PDAC: APE1 BER AND ITS THERAPEUTIC ROLE IN PDAC HOMEOSTASIS

Eyram Kpenu[3,4,5], Silpa Gampala[1], Randall Wireman[1], Melissa Fishel[1], Mark Kelley[2]

[1] *Herman B Wells Center for Pediatric Research, Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN*
[2] *Herman B Wells Center for Pediatric Research, Melvin and Bren Simon Comprehensive Cancer Center, Biochemistry and Molecular Biology, Indianapolis, IN*
[3] *Biochemistry and Molecular Biology*
[4] *Herman B Wells Center for Pediatric Research*
[5] *Melvin and Bren Simon Comprehensive Cancer Center*

Email: *ekpenu@iu.edu*

Pancreatic ductal adenocarcinoma (PDAC) is among the deadliest malignancies due to its resistance to therapeutic strategies and ability to thrive under extreme tumor microenvironment conditions. The cancer's ability to adaptively resist treatments underscores the need for novel and combinatorial therapeutic approaches. Apurinic-apyrimidinic endonuclease/Redox effector factor 1 (APE1/Ref-1) is a critical protein in PDAC pathobiology. APE1 serves two essential functions: as the principal endonuclease in the base excision repair (BER) pathway for DNA repair and as a redox regulator of transcription factors essential for cancer cell proliferation. Targeting APE1's redox function has already demonstrated therapeutic potential. The development of treatments aimed at its BER function, however, has proven more challenging, with limited progress to date. Since standard PDAC treatment regimens involve DNA damaging agents potentially repaired via BER and APE1, we engineered cell lines with knock-in mutations resulting in a reduction of APE1's BER activity while maintaining full redox signaling activity. Biochemical evaluations confirmed a 30-fold reduction in DNA repair capacity. Despite this impairment, the mutant and wild-type cell lines exhibited similar sensitivity to cytotoxic DNA damaging agents in short-term proliferation assays. We posited that the mutant cell lines were relying on compensatory DNA repair pathways which could provide sufficient compensation for the decrease in APE1 activity. We further hypothesized that the cell lines had hidden, long-term deficiencies in DNA damage processing not apparent during the initial stress response. Subsequent long-term functional assays revealed significant vulnerabilities in the mutant cell lines. Colony formation assays revealed that the mutant cell lines exhibit poor long-term survival after short-term exposure to DNA damage. These findings were recapitulated *in vivo* with orthotopic mouse models which demonstrated significant reductions in tumor size and decreased metastasis to the lungs and liver in mutant cell lines compared to wild-type controls. These findings suggest that, while compensatory DNA repair pathways support short-term survival, they may be insufficient to manage DNA damage long-term, resulting in delayed but substantial deficiencies in tumor cell survival. While APE1 is the primary endonuclease in the BER pathway, studies have demonstrated APE1-independent BER in rare contexts. We hypothesize that these APE1-independent BER pathways may function as the compensatory mechanism safeguarding these cells in short-term stress conditions. Characterizing these compensatory pathways will offer a better understanding of how APE1's BER mechanisms contribute to PDAC homeostasis and how these mechanisms can be therapeutically targeted. This work opens the path to enhance standard of care treatments such as FOLFIRINOX.

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

POSTER #121

VITAMIN D BLOCKS PLATINUM-INDUCED OVARIAN CANCER STEM CELL PLASTICITY

Tara X. Metcalfe[1], Sophie Xanders[2], Shu Zhang[3], Heather M. O'Hagan[1], Kenneth P. Nephew[1]

[1] *School of Medicine*
[2] *Jesse H. and Beulah Chanley Cox Scholars Program*
[3] *Department of Biology*

Email: *txmetcal@iu.edu*

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 tumors. CSCs reside in a plastic state, with the ability to convert between non-CSC to CSC. This process of dedifferentiation is thought to continue during tumor development and chemotherapeutic agents such as platinum can exaggerate CSC plasticity. However, the underlying mechanism has not been established. Furthermore, how platinum transforms non-OCSCs into OCSCs to contribute to this subpopulation of cells remains unclear. 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 if platinum induced conversion of non-OCSC to OCSC, ALDH- cells were cultured for 3-10 days after sorting. At timepoints examined, ALDH- cells remained ALDH-, (>99% were ALDH- after 10 days). ALDH- cells were then treated with cisplatin (OVCAR5:12μM,16h, OVCAR3: 15μM 16h), and the percent of ALDH+ cells was measured using flow cytometry. Treatment of cisplatin resulted in conversion of approximately 4% and 10% of ALDH- cells into ALDH+ cells ($p<0.05$) in OVCAR5 and OVCAR3 cells, respectively. Furthermore, converted OCSCs displayed increased ($p<0.05$) expression of stemness genes (BMI1, NANOG, OCT4, SOX2) and spheroid formation compared to parental ALDH+, demonstrating that platinum induced the observed differences in the stemness phenotype. With the goal of targeting key genes and pathways to inhibit platinum-induced OCSC conversion, RNA-sequencing was performed on ALDH- and ALDH+ cells before and after cisplatin treatment. Vitamin D receptor (VDR)/retinoid x receptor (RXR) activation was a major pathway in the platinum-induced converted OCSC transcriptome, which was of interest because VDR signaling plays a role in differentiation, and its key binding partner, RXR and retinoic acid receptor (RAR) are linked to stemness. To determine the effect of vitamin D on OCSC conversion, ALDH- cells were treated with vitamin D for 24h (OVCAR5: 34nM, OVCAR3: 56nM) and/or cisplatin and the ALDH activity was measured with flow cytometry. Vitamin D treatment in combination with cisplatin significantly decreased the platinum-induced percent of converting cells ($p<0.05$). Additionally, vitamin D treatment decreased expression of stemness genes and spheroid formation in both whole cell and sorted cell populations. Currently, we are further investigating the relationship between cisplatin, RAR/RXR signaling and stemness. Collectively, these results demonstrate a role for VDR signaling in platinum-induced OCSC conversion providing further support for the development targeted therapies to block the persistence of OCSCs and ultimately reduce patient mortality.

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

POSTER #122

MYC AND HSF1 CO-AMPLIFICATION IS A BIOMARKER FOR PLK1 AND HDAC INHIBITOR SENSITIVITY IN HIGH-GRADE SEROUS OVARIAN CANCER

Matthew O'Malley[1,8], Imade Williams[2], Haddie DeHart[3], Bobby Walker[4], Vrushabh Ulhaskumar[4], Haimanti Ray[4], Pranav Jothirajah[4], Joe Delaney[5], Kenneth Nephew[6], Richard Carpenter[7]

[1] *Indiana University School of Medicine, Bloomington, Indiana, IU Simon Comprehensive Cancer Center, Indianapolis, Indiana, Bloomington, IN*

[2] *Indiana University, Bloomington, Indiana, Indiana University School of Medicine, Bloomington, Indiana, IU Simon Comprehensive Cancer Center, Indianapolis, Indiana, Indianapolis, IN*

[3] *Indiana University, Bloomington, Indiana, Indiana University School of Medicine, Bloomington, Indiana, IU Simon Comprehensive Cancer Center, Indianapolis, Indiana, Bloomington, IN*

[4] *Indiana University, Bloomington, Indiana, Bloomington, IN*

[5] *Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, South Carolina, Charleston, SC*

[6] *Indiana University, Bloomington, Indiana, Indiana University School of Medicine, Bloomington, Indiana, IU Simon Comprehensive Cancer Center, Indianapolis, Indiana, Department of Anatomy, Cell Biology & Physiology, Indiana University School of Medicine, Bloomington, Indiana, Bloomington, IN*

[7] *Indiana University, Bloomington, Indiana, Indiana University School of Medicine, Bloomington, Indiana, IU Simon Comprehensive Cancer Center, Indianapolis, Indiana, Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Bloomington, Indiana, Bloomington, IN*

[8] *Indiana University, Bloomington, Indiana*

Email: *matomall@iu.edu*

Ovarian cancer is one of the deadliest cancer related malignancies in women. The only FDA approved treatment options for this disease are platinum-based chemotherapies, taxanes, and PARP inhibitors. With these limited therapies, many women experience recurrence of disease after initial benefit which is why the 5-year relative survival time of the disease is only 51%. While platinum-based chemotherapy and taxanes are given to patients regardless of biomarker, PARP inhibitors are reserved for patients with BRCA mutations or other deficiencies in the homologous combination pathway and are the only form of precision-medicine approach for the disease. One thing we have known about high-grade serous ovarian cancer, the most common and deadliest form of ovarian cancer, is that it has a high rate of driver gene amplifications such as *MYC*, *CCNE1*, and *KRAS*. However, this knowledge has yet to be translated into usable therapies for the disease. Our lab has shown that in high-grade serous ovarian cancer, 30% carry co-amplifications of *MYC* and *HSF1*. In these *HSF1* and *MYC* co-amplified ovarian cancer cell lines, loss of either gene product by knockdown decreases proliferation and colony formation ability. In this subpopulation of ovarian cancers with *MYC* and *HSF1* co-amplification, the PLK1 inhibitor volasertib is significantly more effective than in ovarian cancer cells without this co-amplification in disrupting proliferation, colony formation, and spheroid formation ability. In addition to PLK1 inhibition, an epigenetic inhibitor screen indicated that *HSF1-MYC* co-amplified cells were highly sensitive to inhibition of class I histone deacetylates (HDACs). These HDAC inhibitors cause loss of HSF1 expression and MYC protein levels. Consequently, this co-amplification that occurs in approximately one-third of patients can serve as an effective biomarker for sensitivity to several treatments, which could offer patients new therapeutic opportunities in a precision medicine approach.

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

POSTER #123

TOWARDS PERSONALIZED MANAGEMENT OF PANCREATIC INTRADUCTAL PAPILLARY MUCINOUS NEOPLASMS WITH MULTIMODAL ARTIFICIAL INTELLIGENCE

Muhammad Ibtsaam Qadir[1], Jackson Baril[2], Michele Yip-Schneider[2], Duane Schonlau[3], Thi Thanh Thoa Tran[2], C. Max Schmidt[2,4], Fiona Kolbinger[1,5]

[1] *Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN*
[2] *Division of Surgical Oncology, Department of Surgery, Indiana University School of Medicine, Indianapolis, IN*
[3] *Department of Radiology, Indiana University School of Medicine, Indianapolis, IN*
[4] *Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN*
[5] *Regenstrief Center for Healthcare Engineering (RCHE), Purdue University, West Lafayette, IN*

Email: *mqadir@purdue.edu*

According to the international Fukuoka and Kyoto consensus guidelines, the clinical management of pancreatic intraductal papillary mucinous neoplasms (IPMNs), the most common cystic precursor lesion of pancreatic ductal adenocarcinoma, primarily depends on imaging features, cytology, and clinical variables. While sensitive to high-grade or invasive IPMN, these guidelines lack specificity, resulting in surgical overtreatment, i.e., the resection of low-grade IPMN. We propose a clinically applicable multimodal deep learning model to predict the optimal clinical management approach—surgical resection (for high-grade and invasive IPMN) vs. surveillance (for low-grade IPMN)—based on imaging and clinical data. This retrospective study included 180 IPMN patients who underwent surgical resection at Indiana University Health. We developed prediction models for the most appropriate management using individual preoperative magnetic resonance imaging (MRI) sequences (T1 with/without contrast, T2). The multimodal model was developed based on the early fusion of imaging sequences, using a ResNet-34 architecture for imaging data and an XGBoost classifier for clinical features. The multimodal model (F1-score: 0.83, 95% CI: 0.61, 1.00; AUC: 0.93, 95% CI: 0.60, 1.00) outperformed unimodal models trained on T2-weighted MRIs alone (F1-score: 0.59, 95% CI: 0.42, 0.81; AUC: 0.70, 95% CI: 0.44, 0.94), T1-weighted MRIs alone (F1-score: 0.58, 95% CI: 0.40, 0.76; AUC: 0.67, 95% CI: 0.39, 0.92), and T1-weighted contrast-enhanced MRIs alone (F1-score: 0.55, 95% CI: 0.37, 0.74; AUC: 0.47, 95% CI: 0.14, 0.80) on the hold-out test set. The multimodal model also outperformed the stratification performance of Fukuoka criteria (F1-score: 0.54, 95% CI: 0.34, 0.73; AUC: 0.58, 95% CI: 0.31, 0.82) on the holdout test set, showcasing the clinical potential of multimodal AI to personalize management in patients with IPMN. Our findings highlight the added value of multimodal integration, suggesting that multimodal AI models can serve as a valuable diagnostic tool for individualized clinical management of IPMN.

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

POSTER #124

PIM2 KINASE-INDEPENDENTLY REGULATES ITS EXPRESSION IN MULTIPLE MYELOMA

Christopher Schorr[1,2], Daniela Petrusca[1], Joyce Hardwick[1], Louise Carlson[1], Julia Grace Reinke[1], Kelvin Lee[1]

[1] *IU School of Medicine*
[2] *Purdue University Department of Biomedical Engineering*

Email: *cschorr@iu.edu*

Multiple myeloma (MM) remains an incurable hematologic malignancy with poor prognosis due to the inevitable development of drug resistance. Our research investigates a novel regulatory mechanism governing PIM2, an oncogenic kinase overexpressed in MM that critically supports cancer cell survival. Preliminary data from our laboratory demonstrates that PIM2 maintains its elevated expression through a previously uncharacterized **kinase-independent** autoregulatory feedback loop involving the transcription factor MYC. Using chromatin immunoprecipitation assays, we have identified significant MYC occupancy at key regulatory regions of the PIM2 promoter. This occupancy is disrupted by a novel non-ATP competitive PIM2 inhibitor (JP1164) that demonstrates superior efficacy compared to conventional ATP-competitive inhibitors. RNA-sequencing and RT-qPCR analysis reveals that kinase-independent inhibition of PIM2 disrupts MYC-associated gene signatures. Further investigation identified SP1 as an additional transcription factor involved in this regulatory circuit, with SP1 knockdown and inhibition significantly decreasing MM cell viability and PIM2 expression. Exogenous expression studies confirm that both wild-type and kinase-dead PIM2 can enhance endogenous PIM2 expression, supporting our kinase-independent regulatory model. Interestingly, PIM2 and MYC co-localize in the nucleus, suggesting direct cooperation at the chromatin level. Our findings illuminate a critical molecular mechanism underlying PIM2 overexpression in MM and provide a compelling rationale for targeting the PIM2-MYC axis to overcome drug resistance. This work has significant implications for developing novel therapeutic strategies against MM, a disease with high relapse rates and limited treatment options for refractory patients.

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

POSTER #125

A CASE OF HEPATOTOXICITY DUE TO GENE-DRUG INTERACTION IN ROUTINE TREATMENT OF RENAL CELL CARCINOMA

Tayler Smith[1,2], Ashley Springer[2], Yanting Wu[2], Todd Skaar[2,1], Tyler Shugg[2]

[1] *Department of Medical and Molecular Genetics*
[2] *Division of Clinical Pharmacology, Department of Medicine*

Email: *tbs1@iu.edu*

BACKGROUND: Axitinib, a tyrosine kinase inhibitor, and pembrolizumab, an immunotherapy agent, are used as a combination therapy for renal cell carcinoma (RCC). Grade 3-4 hepatotoxicity occurs in 5-10% of cases using this combination therapy. The contributions of the reduced function of the primary metabolism enzymes, Cytochrome P450 3A (CYP3A) to the observed hepatotoxicity remain to be studied.

METHODS: In this case, a 68-year-old male with RCC endured hepatotoxicity upon initiation of axitinib and pembrolizumab combination therapy regimen. The symptoms of the adverse event began on day 10 post-treatment. At that time, the patient visited the emergency room due to nausea/vomiting, diarrhea, and lack of appetite. Laboratory tests for liver function found the patient had elevated aspartate aminotransferase (4.5x), alanine aminotransferase (2.8x), alkaline phosphatase (1.7x), and bilirubin (1.6x). With these results, the decision was made to discontinue the combination therapy. The patient was enrolled in our IRB-approved protocol to investigate genomic causes for exceptional drug response and to avoid further drug-gene interactions. The patient was genotyped using a clinically-validated pharmacogene genotyping panel. Aldy 3.3 was used to extract variants from previously performed germline short-read whole exome sequencing (WES; Ashion Analytics) not included on the genotyping panel for *CYP3A4* and *CYPA5*. Additionally, Oxford Nanopore long-read whole genome sequencing (WGS) was used to phase alleles and check for structural variants in *CYP3A4* and *CYP3A5*.

RESULTS: Genotyping revealed the patient's homozygous loss of function *CYP3A5**3/*3 genotype and heterozygous deceased function *CYP3A4**22 allele. Using Aldy to reanalyze the short-read WES discovered the decreased function variant *CYP3A4**10 (rs4986908). Furthermore, Aldy confirmed *CYP3A4**22 (rs35599367) variant heterozygosity and *CYP3A5**3 (rs776746) variant homozygosity. Long-read WGS demonstrated that *CYP3A4**10 and *22 was in trans orientation and no additional structural variants for the *CYP3A* genes. Altogether, the patient was found to be a poor metabolizer for CYP3A4 and CYP3A5substrates given their *CYP3A4* *10/*22 and *CYP3A5**3/*3 genotype.

CONCLUSION: The patient's dual poor metabolizer status for CYP3A4 and CYP3A5 likely contributed to the hepatoxicity observed in response to axitinib and pembrolizumab combination therapy leading to the discontinuation of therapy. Future analysis of the impact of the *CYP3A4* *10 and *22 alleles on axitinib metabolism should be conducted.

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

POSTER #126

ELUCIDATING ISL1 ROLE AS A PREDICTIVE BIOMARKER FOR LURBINECTEDIN RESPONSE IN SMALL CELL LUNG CANCER

Olivia Terry[1], Katherine Minton[1], Tianhao Zhou[1], Paresh Kumar[1], Erin Hanna[1], Hilal Ozakinci[2], Theresa Boyle[3], John Koomen[4], Michael Shafique[4], Misty Shields[1]

[1] *Department of Medicine, Division of Hematology/Oncology, Indiana University Melvin and Bren Simon Comprehensive Care Center, Indiana University School of Medicine*
[2] *Department of Thoracic Oncology, Moffitt Cancer Center*
[3] *Department of Pathology, Moffitt Cancer Center*
[4] *Molecular Oncology and Molecular Medicine Program, Moffitt Cancer Center*

Email: *octerry@iu.edu*

Background:

Small cell lung cancer (SCLC) is an aggressive neuroendocrine malignancy known for its initial chemosensitivity, followed by relapse. In 2020, lurbinectedin received accelerated approval for relapsed SCLC. Real-world data suggests ~4 cycles prior to progression of disease. Lurbinectedin uniquely binds to guanines in the minor groove of DNA, stalls RNA polymerase II, and changes the tumor microenvironment. In our previous research, SLFN11 failed to predict lurbinectedin response in patient samples. Proteomics identified ISL1 as top "hit" associated with durable lurbinectedin responses. ISL1 is a LIM transcription factor required for tracheobronchial differentiation. ISL1 is overexpressed in multiple cancer types. Here, we investigate the molecular impact of ISL1 for lurbinectedin response in SCLC.

Methods:

Cell Viability Assay: A total of 1,000 cells were incubated for 24 hours (h), prior to lurbinectedin (0.01-1 nM) for 72 h (or DMSO) for luminescence.

Treatment: A total of 1 x 10^6 cells were incubated for 24 h, prior to 0.1 nM lurbinectedin (or DMSO) and harvested at 24h.

ISL1 Knockdown: Reverse transfection of 50 nM small interfering RNA (siRNA) against ISL1 (or control) was introduced in 3 x 10^5 SCLC and harvested at 72 h.

Quantitative PCR: RNA was extracted using RNeasy Mini Kit (Qiagen) and quantified. cDNA was prepared. Taqman probes: ISL1, SOX5, ASCL1, NeuroD1, Vinculin, and GAPDH.

Western Blot: Prepared lysates were run on Bio-Rad Mini-PROTEAN TGX gel and Trans-Blot Turbo Transfer System was used. Membranes were incubated at room temperature for 5 minutes in EveryBlot Blocking Buffer, 1:1000 primary antibody (Ab) for 0.5 h, 1:5000 secondary Ab in EBB for 0.5 h. Antibodies: ISL1, cyclophilin B, vinculin, and b tubulin.

Results:

ISL1 protein expression varies among neuroendocrine carcinomas. ISL1 appears to be expressed in a background of ASCL1, compared to other neuroendocrine subtypes. Lurbinectedin response is differential across SCLC and correlates with ISL1 status in ASCL1+ SCLC (R^2 = 0.81), in conjunction with patient samples. Lurbinectedin treatment results in decreased expression of ISL1, SOX5, ASCL1, SIX1, SIX4 and confirmed by siRNA to be directed via ISL1. Transient knockdown of ISL1 results in an increase in the IC_{50} to lurbinectedin.

Conclusion:

ISL1 protein expression is a novel biomarker for predicting response to lurbinectedin in SCLC. ISL1 is expressed in a subset of SCLC, particularly ASCL1+ subtypes and directly impacts the expression of ASCL1 and SOX5 in a positive feedback mechanism. We hypothesize ISL1 regulates SOX5 and ASCL1 to cooperate in bending of the minor groove to permit lurbinectedin incorporate into the SCLC DNA, resulting in cell death and exquisite sensitivity to the drug. Mechanistic studies assessing ISL1's role to induce DNA damage, ISL1's interaction with SOX5 at the minor groove of DNA, and elucidating ISL1's role to dictate neuroendocrine subtype plasticity are underway.

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

POSTER #127

DNA METHYLTRANSFERASE INHIBITION PREVENTS PLATINUM-INDUCED OVARIAN CANCER STEM CELL ENRICHMENT BY POTENTIALLY ALTERING STAT3 AND NF-KB ACTIVATION

Truc T. Vuong[3], Rena Y. Han[1], Heather M. O'Hagan[2]

[1] Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN, 47405, Bloomington, IN
[2] Cell, Molecular and Cancer Biology Graduate Program, Indiana University School of Medicine, Bloomington, IN, 47405, Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN, 47405, Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, 46202, Bloomington, IN
[3] Cell, Molecular and Cancer Biology Graduate Program, Indiana University School of Medicine, Bloomington, IN, 47405

Email: *ttvuong@iu.edu*

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) is enriched in recurrent tumors and drives 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. RNA-Seq analysis of OC cells treated with platinum with or without DNMTi pre-treatment identified 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. Our preliminary data showed that in response to platinum and/or DNMTi, NF-kB and STAT3 were activated differently, and activation of both transcription factors was required for platinum-induced OCSC enrichment. 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. *Together, I hypothesize that the combination of DNMTi and platinum induces NF-kB and decreases STAT3 activation, altering NF-kB genomic binding and leading to changes in transcription of target genes, thus blocking platinum-induced OCSCs*. To test this hypothesis, a constitutively active STAT3 mutant will be utilized to examine whether DNMTi prevents platinum-induced OCSC enrichment through decreasing STAT3 activation. Additionally, CUT&RUN assay was used to investigate the binding of NF-kB and STAT3 to the genome after the different treatments. In addition to altering NF-kB and STAT3 activation, I found that *TERT* expression decreased when treating OC cells with DNMTi and that *TERT* was necessary for platinum-induced OCSC enrichment. Interestingly, *TERT* expression was the highest in OCSCs resulted from treating non-OCSCs with platinum. *These preliminary data suggest that TERT is a potential candidate for further examination*. By elucidating how DNMTi 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 #128

MODULATION OF THE TUMOR MICROENVIRONMENT BY RADIATION THERAPY TO ENHANCE IMMUNE ACTIVITY IN GLIOBLASTOMA

Chandler Zaugg[1], Jessica Veenstra[1], MacKenzie Coon[1], Justin Geise[1], Judith Rivera[2], Matthew Scarpelli[1]

[1] *School of Health Sciences, Purdue University, West Lafayette, Indiana, USA*
[2] *Department of Radiation Oncology, Indiana University, Indianapolis, Indiana, USA*

Email: *czaugg@purdue.edu*

Purpose: Glioblastoma (GBM) is among the most aggressive and treatment-resistant cancers due to its immunosuppressive tumor microenvironment. Immunotherapy holds promise for GBM treatment, but its efficacy is constrained by limited immune activity within the tumor. This study investigates how radiation therapy (RT) modulates immune cell populations in the GBM microenvironment to identify RT regimens that prime the tumor for adoptive immunotherapy. By elucidating immune response timing, we aim to establish optimal conditions for combining RT with immunotherapy to improve clinical outcomes.

Methods: Thirty-four mice (18 female, 16 male) were orthotopically injected with GL-261 glioma cells to establish a GBM model. Immune cells were evaluated in female mice across three time points (24, 72, and 144 hours) post-RT. Male mice were assessed at 72 hours post-RT. Immune cells were quantified using immunohistochemistry and included natural killer cells (CD335+), neutrophils (Ly6G+), T-cells (CD3+), and macrophages (CD68+). Pairwise comparisons assessed RT-induced immune cell changes relative to control cohorts without RT.

Results: In female mice, macrophage populations increased significantly at 72 hours ($p=0.01$) and 144 hours ($p=0.002$) after a 20-Gy radiation dose, suggesting a central role in immune priming. T-cells significantly decreased at 24 hours after 20-Gy ($p=0.03$), indicating potential early immunosuppression of these cells, but recovered by 144 hours. In contrast, natural killer cells and neutrophils showed no significant changes, highlighting cell-specific responses to RT. In male mice, macrophage populations increased significantly at 72 hours after 20-Gy ($p=0.04$), while other immune populations showed no significant changes.

Conclusion: This study demonstrates RT's potential to modulate immune populations and create a therapeutic window for adoptive immunotherapy in GBM. There were significant increases in macrophage populations 72 hours post-RT, indicative of a potential target for combination immunotherapy. By identifying optimal time points for immune infiltration, this work provides a framework for designing synergistic RT-immunotherapy regimens.

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

POSTER #129

S-ADENOSYL-METHIONINE (SAM) SYNTHESIS PROTECTS OVARIAN CANCER FROM CHEMOTHERAPY INDUCED DNA DAMAGE AND CONTRIBUTES TO CANCER STEM CELL ENRICHMENT

Shu Zhang[4], Tara X. Metcalfe[1], Fu Zhen[2], Yanchi Zhou[1], Heather M. O'Hagan[3], Kenneth P. Nephew[3]

[1] *School of Medicine, Indiana University-Bloomington, Bloomington, IN*
[2] *Bioinformatics and Biostatistics Core, Van Andel Institution, Grand Rapids, MI*
[3] *School of Medicine, Indiana University-Bloomington, Bloomington, IN*
[4] *Department of Biology, Indiana University-Bloomington*

Email: *szh1@iu.edu*

High-grade serous ovarian cancer (HGSOC) is a deadly gynecological malignancy characterized by tumor recurrence and chemoresistance to first-line therapies, including platinum (Pt)-based chemotherapy. We and others have shown that Pt-induced increase in ovarian cancer stem cells (OCSCs) contributes to disease relapse. Methionine (Met) metabolism has been shown to maintain CSC phenotypes, but the role of Met in Pt-enriched OCSCs remains unclear. HGSOC cell lines were sorted using FACS into OCSCs and non-CSCs. Expression of methionine adenosyltransferase 2A (MAT2A), the enzyme converting Met to S-adenosyl-methionine (SAM), was higher ($p<0.01$) in OCSCs compared to non-CSCs. Supplementation of extracellular SAM (50mM) increased ($p<0.05$) the percentage of OCSCs, whereas inhibiting MAT2A by siRNA knockdown or the activity inhibitor effectively prevented ($p<0.05$) the cisplatin (IC50, 16hr) induced increase in both OCSCs population and spheroid formation, demonstrating the importance of SAM biosynthesis in OCSC enrichment. The combination treatment of cisplatin and siMAT2A enhanced ($p<0.01$) DNA damage and apoptotic cell death compared to either treatment alone. Mechanistically, siMAT2A impaired ($p<0.05$) functions of proteins involved in the DNA damage response and repair, including yH2Ax, ATM, ATR, Chk1 and FANCD2. MAT2A knockdown also suppressed ($p<0.05$) the cisplatin-induced upregulation of cyclin E/CDK2 which consequently increased the percentage of cells by-passing the cell cycle checkpoint for DNA repair. Furthermore, siMAT2A increased ($p<0.05$) the binding of SAM sensor protein BMT2 on GATOR1, the negative regulatory complex of mTORC1, attenuating p70-S6 kinase and potentially contributing to the downregulation of cyclin E. Notably, the effects of siMAT2A were independent of the functionality of Met salvage enzyme methylthioadenosine phosphorylase. As SAM is a universal methyl donor for various methylation processes and given the established link between OCSC enrichment and aberrant DNA methylation, we used Infinium MethylationEPIC array v2.0 BeadChip to investigate genome-wide changes in DNA methylation induced by cisplatin and/or siMAT2A. Cisplatin alone increased hypermethylated regions compared to the control. The combination of cisplatin and siMAT2A resulted in a unique pattern of hypomethylation compared to cisplatin only, demonstrating a critical role of SAM synthesis in cisplatin-altered DNA methylation dynamics. Enrichment analysis identified genes associated with stemness, differentiation, and cell death. Collectively, our study demonstrates a new role of SAM synthesis in Pt-induced DNA damage repair and OCSCs enrichment, providing a potential therapeutic target for improving chemotherapy outcomes and preventing OCSC enrichment in ovarian cancer.

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

POSTER #130

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

Yanchi Zhou[2], Kenneth Nephew[1]

[1] *Medical Sciences, Indiana University School of Medicine, IU Melvin and Bren Simon Comprehensive Cancer Center, Bloomington, IN*

[2] *Medical Sciences, Indiana University School of Medicine*

Email: *yanczhou@iu.edu*

Poly (ADP-ribose) polymerase inhibitors (PARPis) are an important treatment modality for homologous recombination deficient (HRD) cancers, including high grade serous ovarian cancer (HGSOC). PARP 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 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 and HRD HGSOC cell lines. Knockout or siRNA knockdown of HOTAIR sensitized ($p<0.05$) HGSOC cell lines to PARPis (olaparib, talazoparib). HOTAIR upregulated the expression of checkpoint kinase 1 (CHK1), a key kinase involved in DNA damage response. Treatment with talazoparib (5μM, 72hrs) increased ($p<0.05$) expression of CHK1, while HOTAIR depletion with paired CRISPR gRNAs decreased ($p<0.05$) CHK1 activation by PARPi. In contrast, overexpression of HOTAIR enhanced ($p<0.05$) talazoparib-induced activation of CHK1. It has been reported that CHK1 inhibition converted PARPi-insensitive cells to PARPi-sensitive cells and, furthermore, that CHK1 interacts with the DNA repair protein RAD51 which was necessary to promote the reversal of stalled replication forks and protect the reversed fork from degradation. Thus, we hypothesized that HOTAIR contributes to PARPi resistance in HGSOC cell lines by regulating CHK1 and CHK1-mediated replication forks stabilization and DNA damage response. We demonstrated that RAD51 expression was reduced ($p<0.05$) in HOTAIR depletion 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 examined the effect of PARPi on DNA damage using the comet assay. In HOTAIR overexpressed HGSOC cells, PARPi-induced DNA damage was reduced ($p<0.001$), based on comet tail moment. As no effect of HOTAIR on CHK1 transcription was observed, we investigated the mechanism of HOTAIR regulating CHK1 by using the cycloheximide chase assay and assessing CHK1 protein stability. HOTAIR depletion increased ($p<0.05$) degradation of CHK1, and HOTAIR overexpression stabilized CHK1 protein. Collectively, these findings suggest that HOTAIR contributes to CHK1 s**tabilization**, promotes replication fork protection and DNA damage repair in HGSOC and in turn HOTAIR contributes to PARPi resistance, regardless of HR status.

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

POSTER #131

TARGETING RNASE L TO ENHANCE IMMUNTHERAPY IN TRIPLE-NEGATIVE BREAST CANCER

Grace Xiyu Wang[1,3], Lizzie Adams[1,4], Qingfei Wang[2,5], Tao Yu[2,5], Mateusz Opyrchal[2,5]

[1] *School of Medicine, Indianapolis, IN*
[2] *Department of Hematology and Oncology, Indianapolis, IN*
[3] *MMGE*
[4] *Department of Hematology and Oncology*
[5] *School of Medicine*

Email: *xw73@iu.edu*

Treatment options for triple-negative breast cancer (TNBC) have historically been limited. Despite TNBC's immunogenic molecular features, such as high PD-L1 expression, elevated tumor mutational burden, and increased tumor-infiltrating lymphocytes, which are often associated with improved responses to immune checkpoint blockade (ICB), ICB monotherapy demonstrates only modest efficacy in TNBC. Robust cytotoxic T cell (CTL)-mediated immune responses depend on effective tumor antigen presentation, which remains a key barrier to ICB success in solid tumors. Enhancing tumor antigen presentation therefore represents a promising strategy to improve ICB efficacy in TNBC.

Using a murine whole-kinome CRISPR library in the E0771-Ova TNBC model, our laboratory identified twenty-six top-ranked candidate genes that regulate tumor antigen presentation. Among these, RNase L depletion significantly increased antigen presentation in E0771-Ova cells, potentially by enhancing translational capacity and the production of tumor-associated antigens. While RNase L is well-studied in the context of antiviral and anti-inflammatory responses, its role in tumor biology and immunotherapy remains largely unexplored.

We hypothesize that RNase L depletion enhances translational capacity in TNBC cells, leading to increased production of tumor-associated antigens and a strengthened anti-tumor immune response. In a syngeneic breast cancer mouse model, RNase L-depleted TNBC cells exhibited markedly reduced tumor growth and increased CD8+ T cell infiltration in the tumor microenvironment. Moving forward, we aim to elucidate the mechanisms by which RNase L depletion affects tumor progression and evaluate its therapeutic potential in combination with immune checkpoint blockade.

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

POSTER #132

EVALUATING CLINICAL OUTCOMES OF VMAT TOTAL BODY IRRADIATION IN BONE MARROW TRANSPLANT RECIPIENTS

Sidra Ahmad[5], Samantha Freije[1], Colin Huang[2], Jodi Skiles[2], April Rahrig[2], Toshihiro Onishi[2], Courtney Spiegel[2], Yong Zang[3], LaKeisha Boyd[4], Naoyuki Saito[1]

[1] *Indiana University School of Medicine, Department of Radiation Oncology, , IN*
[2] *Indiana University School of Medicine, , IN*
[3] *Department of Biostatistics, Indiana University School of Medicine, , IN*
[4] *Indiana University, , IN*
[5] *Indiana University School of Medicine*

Email: *sidtahmad@gmail.com*

Purpose/Objective: Total body irradiation (TBI) is an integral part of conditioning for bone marrow transplant (BMT) patients. Traditionally, TBI is delivered using a two-beam technique, but volumetric modulated arc therapy (VMAT) offers an alternative for intensity-modulated TBI, allowing precise dosing to organs at risk while ensuring adequate dose to targets. This study evaluates outcomes of patients receiving VMAT-TBI at our center.

Materials/Methods: Outcome data was collected from 9 patients who received 12 Gy VMAT-TBI as part of their myeloablative conditioning for allogeneic BMT. Descriptive statistics were used to analyze the data, which were then compared to patients treated with conventional TBI at our institution.

Results: Nine patients (seven with ALL, one with CML, and one with MPAL) received 12 Gy VMAT TBI. CIBMTR risk scores were 0 (five patients), 1 (two patients), and 3 (two patients). Median age was 14 years (6-24) at time of treatment. Median follow-up was 27 months. Five patients were alive at end of follow-up. Mean time to death for the four deceased patients was 291.75 days. Mean neutrophil and platelet engraftments were 20.89 days (12-27) and 25 days (17-38). Infections included Grade 1 rhinovirus and EBV in the same patient at days 48 and 64, and Grade 1 acute GVHD at day 41 in one patient. Grade 2 rhinovirus occurred in one patient at day 6. Grade 2 acute GVHD occurred in 3 patients (days 22, 32, 45). Forty-one Grade 3 toxicities were observed in 8 patients: CMV (days 49 and 50); cytokine release syndrome (day 4); hypoxia (days 10 and 24); GI hemorrhage (days 7, 8, 9, 14, 19, and 56); fluid overload (days 2 and 15); abdominal pain (day 9); bacteremia (days 0, 3, and 9); hypotension (days 12, 56, and 61); cellulitis (day 18); hypertriglyceridemia (day 7); hyperglycemia (day 35); TMA (day 37); BK virus (day 38); epistaxis (days 3, 7, 11, 17, and 19); oral mucositis (days 2, 3, 4, 6, 6, and 10); diarrhea (days 4, 5, 6, and 18); and acute GVHD (day 30). Chronic GVHD in the mouth, eyes, and liver was seen in one patient (day 183). Twelve Grade 4 toxicities were seen in four patients: sepsis (day 12); pulmonary hemorrhage (day 11); AKI (days 12, 36, and 45); sinusoidal obstruction syndrome (days 9 and 14); hypertriglyceridemia (day 37); encephalopathy (day 35); hypotension (days 36 and 44); and fungemia (day 54). One patient died from Grade 5 sepsis (day 60).

Conclusion: Clinical outcomes of patients receiving VMAT-TBI appear not dissimilar to those treated with conventional TBI at our institution. In addition to these findings, we will present pre- and post-SCT EKG, echocardiogram, and PFT data, along with a comparative analysis of historical data from conventional TBI patients.

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

POSTER #133

SGLT2 INHIBITOR USE FOR CARDIAC PROTECTION IN A PATIENT WITH OSIMERTINIB-RESPONSIVE ADVANCED EGFR-POSITIVE LUNG CANCER

Aditya Belamkar[3], Marwan Mounayar[1], Suparna Clasen[2]

[1] *Indiana University Ball Memorial Hospital Cancer Center, Indiana University School of Medicine, Muncie, IN*
[2] *Indiana University School of Medicine, Indianapolis, IN*
[3] *Indiana University School of Medicine*

Email: *abelamka@iu.edu*

Background

Osimertinib is a first-line therapeutic agent for non-small cell lung cancer (NSCLC) with specific EGFR mutations. It offers improved outcomes and the ability to manage resistant mutations and brain metastases. However, use requires close monitoring of cardiotoxicity, including heart failure (HF), arrhythmias, and QT prolongation.

Case

One month after starting osimertinib for metastatic EGFR+ NSCLC, an 85-year-old man presented in decompensated HF with preserved LVEF and prolonged QTc. Osimertinib was held while undergoing HF optimization, and clinical status improved. After restarting therapy, LVEF was found to be reduced. Osimertinib was held, and losartan and metoprolol succinate were initiated to maximally tolerated doses. With each attempt at reintroduction, LVEF declined, suggesting osimertinib-induced cardiomyopathy. Empagliflozin was started for cardioprotection before another rechallenge of osimertinib. At 12-month follow-up, the patient remained stable on electrocardiographic and echocardiographic examinations.

Discussion

SGLT2 inhibitors are a powerful tool for HF; however, they may have a secondary benefit in mitigating cardiotoxicity. Previous studies highlighted SGLT2 inhibitors' anti-inflammatory mechanisms in animal models and patients treated with anthracyclines and trastuzumab. In our case, traditional cardioprotective agents were ineffective, and limited blood pressure precluded sacubitril-valsartan or spironolactone. While SGLT2 inhibitors are not yet well established for cancer therapy-related cardiomyopathy, current HF guidelines and emerging research support their potential use.

Conclusion

We recommend osimertinib discontinuation followed by guideline-directed HF management, focusing on initiating an SGLT2 inhibitor, secondary prevention through routine echocardiography, and cautious reintroduction of osimertinib upon cardiac stabilization. This case underscores the importance of proactive management strategies to mitigate cardiovascular risks associated with osimertinib while ensuring continued oncologic therapeutic efficacy.

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

POSTER #135

SOCIAL DETERMINANTS OF POSTOPERATIVE LENGTH OF STAY FOLLOWING ENDOSCOPIC ENDONASAL PITUITARY SURGERY

Anoop Chinthala[1], Chad Purcell[2], Bobby Woodburn[1], Ajay Patel[1], Matthew Pease[1], Angela Richardson[1], Satyan Sreenath[2], Mitesh Shah[1], Joao Paulo Almeida[1]

[1] *Indiana University School of Medicine; Department of Neurological Surgery*
[2] *Indiana University School of Medicine; Department of Otolaryngology*

Email: *aschinth@iu.edu*

Background: Socioeconomic disparities are increasingly recognized as contributors to variable healthcare outcomes, including hospital length of stay (LOS). This study investigates how social factors, including the Social Vulnerability Index (SVI) and Area Deprivation Index (ADI), influence postoperative LOS in patients undergoing endoscopic endonasal pituitary surgery.

Methods: A retrospective cohort who underwent endoscopic transsphenoidal resection of pituitary adenoma or cyst was analyzed from 2021-2024. SVI and ADI scores were collected based on ZIP codes. LOS was compared across sociodemographic variables using Wilcoxon rank-sum and Kruskal-Wallis tests. A multivariable linear regression assessed the relationship between LOS and both SVI and ADI percentiles, with $p < 0.05$ considered statistically significant.

Results: We identified 159 patients with an average age of 53 years; 48% female and 52% male. Racial distribution was predominantly White (77%), followed by Black (17%) and Asian (3.1%). The average SVI was 0.60 ± 0.25 and average ADI was in the 63rd percentile ± 23, indicating a medium to high vulnerable and deprived cohort. Average postoperative LOS was 4.58 ± 3.32 days. Comparative analysis demonstrated that females had a longer length of stay than males ($p = 0.010$), patients that were unemployed at time of admission had a longer length of stay to those employed ($p = 0.021$), and patients without a primary care physician at time of admission had a longer length of stay ($p = 0.031$). In regression analysis, higher ADI percentiles were significantly associated with increased LOS ($\beta = 0.031$, $p = 0.016$), while SVI was not a significant predictor ($p = 0.90$).

Conclusion: Socioeconomic disadvantage, particularly higher ADI percentiles, was associated with longer postoperative length of stay after pituitary surgery. Factors such as unemployment and lack of a primary care physician also contributed to prolonged hospitalization. These findings highlight the impact of social determinants on surgical recovery. Addressing these disparities may improve outcomes and reduce healthcare costs.

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

POSTER #136

ANALYSIS OF RURAL-URBAN TREATMENT ACCESS PATTERNS IN PRIMARY BRAIN TUMOR CARE: INFRASTRUCTURE DISTRIBUTION AND CARE DELIVERY MODELS: A COMPREHENSIVE REVIEW

Anoop Chinthala[3], Barnabas Obeng-Gyasi[1], Halie Szilagyi[1], Trenton Line[1], Kathryn Nevel[2], Piiamaria Virtanen[1], Angela Richardson[1]

[1] *Indiana University School of Medicine; Department of Neurological Surgery, Indianapolis, IN*
[2] *Indiana University School of Medicine; Department of Neurology, Indianapolis, IN*
[3] *Indiana University School of Medicine; Department of Neurological Surgery*

Email: *aschinth@iu.edu*

Background: Primary brain tumors require complex neurosurgical care and coordinated treatment approaches. Geographic location and healthcare infrastructure distribution can significantly impact access to care delivery, particularly affecting treatment initiation and completion rates between rural and urban populations.

Methods: A comprehensive literature review was conducted using OVID, Cochrane Library, and EMBASE databases (inception through November 2024). Key search terms included "primary brain tumors," "surgical treatment," "geographic access," and "healthcare infrastructure." Articles were independently screened using predetermined criteria focusing on treatment access patterns and care delivery models.

Results: Analysis revealed significant geographic variation in care access, with nonmetropolitan patients 7% less likely to receive optimal surgical treatment. Only 8.7% of clinical trial sites were located in rural counties, with documented provider distribution gaps creating healthcare "coldspots." Travel distance to treatment centers averaged >75 miles for rural populations compared to <25 miles for urban residents. Implementation of hybrid care delivery models combining telemedicine with in-person visits showed promise in addressing access barriers.

Conclusion: Geographic factors significantly impact access to primary brain tumor treatment. Evidence-based solutions including strategic infrastructure development and technology-enabled care delivery models show potential for optimizing resource distribution and improving care access patterns. Future healthcare system development requires systematic evaluation of resource allocation strategies and care delivery optimization.

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

POSTER #137

CHARACTERISTICS AND OUTCOMES OF PATIENTS TREATED WITH ENFORTUMAB VEDOTIN (EV) /PEMBROLIZUMAB FOR METASTATIC UROTHELIAL CARCINOMA: THE IMPACT OF DURATION OF EV ON SURVIVAL OUTCOMES

Derek Krismer[1]

[1] *Indiana University School of Medicine*

Email: *dkrismer@iu.edu*

Introduction:

Enfortumab vedotin (EV) with pembrolizumab (pembro) is a new standard for patients (pts) with locally advanced (LA) or metastatic urothelial carcinoma (UC). While the duration of pembro in EV-302 was 2 years, EV was continued indefinitely until unacceptable toxicity or disease progression, though EV side effects often limit duration. Here, we evaluate the characteristics and survival outcomes in pts treated with varying durations of EV.

Methods:

The prospectively maintained Indiana University UC database was queried for pts with LA or metastatic UC that were treated with EV/pembro. Pts were categorized into three groups based on the number of cycles of EV received (0-4 cycles, 5-9 cycles, or >9 cycles). Comparisons between groups were done using Chi-square or Fisher's Exact test for categorical variables or Kuskal-Wallis test for continuous variables. The Kaplan-Meier method was used to analyze progression free survival (PFS) and Overall Survival (OS).

Results:

45 pts with LA/metastatic UC treated with EV/Pembro were identified. The median age at diagnosis was 70 yrs (46.2-89.1). 19 pts (42.2%) received 0-4 cycles of EV, 16 pts (35.6%) received 5-9 cycles of EV, and 10 pts (22.2%) received >9 cycles of EV. Predominant histology was urothelial (66.7%), mixed (17.8%), poorly differentiated (8.9%) and other (6.6%). 21 pts (46.7%) had metastatic disease at diagnosis while 24 pts (53.3%) were at relapse. EV was dose reduced in 27 pts (60%). Reasons for dose reduction were neuropathy (40.7%), rash (29.6%), poor PS (14.8%), and other (14.9%). In 10 pts (22.2%), EV was stopped because of toxicity while pembro was continued. Reasons for stopping EV were neuropathy (4 pts), rash (2 pts), fatigue (2 pts), and other (2 pts).

At a median follow up time of 10.7 months, median PFS in pts who received 0-4 cycles of EV was 3.5 mos (1.7-7.3), vs. 7.7 mos (4.7-NE) for 5-9 cycles vs. NE (7.2-NE) for >9 cycles of EV ($p<0.001$). Median OS in pts who received 0-4 cycles of EV was 8.8 mos (3.0-NE), vs. NE (8.7-NE) for 5-9 cycles vs. 30.3 mos (13.1-30.3) for >9 cycles of EV ($p=0.001$). To account for the impact of progression being the reason for fewer EV cycles and thus poorer survival, pts who stopped EV because of disease progression (n=14) were then excluded. Median PFS in pts who received 0-4 cycles of EV was 3.7 mos (2.4-9.2) vs. NE (4.7-NE) for 5-9 cycles vs. NE (11.3-NE) for >9 cycles($p=0.0004$). Median OS in the same cohort was 9.8 mos (3.0-NE) in pts who received 0-4 cycles vs. NE (8.7-NE) for 5-9 cycles vs. NE (13.8-NE) for >9 cycles of EV ($p=0.10$).

Conclusions:

In this real-world cohort of pts with LA/metastatic UC treated with EV/pembro, the majority required dose reduction of EV. Almost a quarter of pts had to stop EV early because of toxicity but continued pembro. Larger populations are needed to evaluate the impact of this EV de-escalation on survival outcomes.

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

POSTER #138

DIAGNOSTIC CHALLENGES IN HPV-INDEPENDENT VULVAR PATHOLOGY: A CASE REPORT OF DIFFERENTIATED VULVAR INTRAEPITHELIAL NEOPLASIA (DVIN) IN THE SETTING OF LICHEN SCLEROSUS

Josie McQuillan[3], Caesy Woods[1], Elaina Lewis[1], Mary Skelly[1], Rachel Kowal[2]

[1] *Indiana University School of Medicine, Bloomington, IN*
[2] *Indiana University School of Medicine, Division of Dermatopathology, Indianapolis, IN*
[3] *Indiana University School of Medicine*

Email: *jmcquil@iu.edu*

Background: Vulvar neoaplasia is classified by human papillomavirus infection status. Detecting precursor lesions (vulvar intraepithelial neoplasia - VIN) is key to preventing invasive carcinoma. High-risk HPV-associated VIN or high-grade squamous intraepithelial lesion (HSIL) typically affects younger patients and shows classic histology with p16 overexpression. In contrast, HPV-independent or differentiated VIN (dVIN) mainly affects older women, often with chronic inflammatory dermatoses like lichen sclerosus. dVIN shows p16 negativity and distinct p53 patterns, with variants like verruciform/acanthotic VIN (vaVIN) lacking p53 mutations, complicating detection. Notably, HPV-independent VIN has a worse prognosis than HSIL.

Case Description: An 81-year-old woman presented with vulvar swelling, redness, and pain. Clinical exam revealed erythema from the vulva to the perineum and a 1 cm ulcerated right vulvar lesion. Biopsy showed dVIN with negative p16 and p53 overexpression, along with lichen sclerosus. Wide local excision showed a 1.2 cm well-differentiated, HPV-independent SCC with positive margins near the urethra. Postoperative PET/CT showed no metastasis. Due to positive margins and urethral proximity, chemoradiation was started but discontinued after 3 weeks due to unrelated ICU-level illness.

Clinical Significance: This case shows the importance of early screening for HPV-independent VIN, especially in those with lichen sclerosus. Pathologists should be vigilant in detecting VIN to prevent progression to vulvar SCC. Clinicians should screen post-menopausal, non-sexually active women, who may not report symptoms or seek screening.

Conclusions: The subtle histology and overlap with chronic inflammatory dermatoses make HPV-independent VIN difficult to detect clinically and histologically. Routine screening and a low threshold for biopsy, especially in those with lichen sclerosus, are vital for better outcomes. Given these diagnostic challenges, increased clinician and pathologist awareness is essential for preventing vulvar SCC.

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

POSTER #139

UNCOVERING EARLY MARKERS OF MALIGNANT TRANSFORMATION IN NF1-ASSOCIATED PERIPHERAL NERVE SHEATH TUMORS: AN INTEGRATED ANALYSIS OF IMMUNE MICROENVIRONMENT AND RADIOLOGIC FEATURES

Janak Mukherji[1], Carina Dehner, Kylee Brewster, Henry Mang, Roman Shrestha, Rachel Kowal, Emily White, Dana Mitchell, D Wade Clapp, Steven Rhodes

[1] *Indiana University School of Medicine*

Email: *jmukher@iu.edu*

Neurofibromatosis type 1 (NF1) is a multisystem, autosomal dominant disorder that affects approximately 1 in 3000 newborns. Plexiform neurofibromas (PN) are present in about half of cases and cause significant morbidity due topain, disfigurement and motor dysfunction. PN have a lifetime risk of ~10% of transforming into malignant peripheral nerve sheath tumor (MPNST), a highly aggressive and metastatic form of soft tissue sarcoma with poor survival. The lack of reliable biomarkers to identify PN at high risk of undergoing malignant transformation poses a significant challenge. Our recent research has revealed that a subset of benign-appearing PN and premalignant atypical neurofibromas exhibit deregulated signatures of immune surveillance and T cell infiltration that preceded malignant transformation. To identify early markers of disease progression, we are conducting a comprehensive analysis ofarchival plexiform and atypical neurofibroma specimens, comparing lesions that are either associated or not associated with malignant transformation. Our study involves a three-step approach: 1) Clinical data (including tumor location, symptoms, treatment history, indication for surgical resection, etc.) and imaging features (MRI, PET/CT, PET/MRI) were extracted retrospectively from 175 clinical cases. 2) Histopathologicalevaluation is in process for ~125 specimens withimmunohistochemical stainingto assess markers of tumor progression (p16, p53, CD34, H3k27me3,Ki67), T cell infiltration (CD3, CD4, CD8, FOXP3), and Schwann cell lineage (S100, SOX10). 3) Blinded reanalysis of imaging data (MRI, PET/CT, PET/MRI) to correlateradiologic features withlinked clinical and molecular data. This integrated analysisrepresents a critical first step to developing molecular and radiologic diagnostic tools to guide risk adapted care in patients with NF1-associated PN. The findings will further inform hypothesis-driven research in preclinical models of NF1-tumorigenesis to validate these biomarkersand identify novel treatment approaches for individuals affected by these rare but devastating tumors.

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

POSTER #140

ANTI-TUMOR EFFECTS FOLLOWING BET INHIBITION IN PRECLINICAL MODELS OF PEDIATRIC AND AYA OSTEOSARCOMA

Ryli Justice[1,2], Erika Dobrota[2], Rada Malko[1,2], Harlan Shannon[2], Keiko Kreklau[2], Melissa Trowbridge[1], Kathryn Coy[1], Felicia Kennedy[1], Melinda Ervin[1], Anthony Sinn[1], Christopher Davis[3], Steve Angus[1,2], M. Reza Saadatzadeh[1,2], Pankita Pandya[1,3], Karen Pollok[1,2,3]

[1] *Indiana University Simon Comprehensive Cancer Center*
[2] *Department of Pediatrics, Herman B Wells Center for Pediatric Research*
[3] *Department of Pediatrics, Hematology/Oncology*

Email: *rejustic@iu.edu*

Osteosarcoma (OS) is the most common primary malignant bone sarcoma in pediatric, adolescent, and young adult patients, with a five-year survival rate of <30%. This is due, in part, to genomic complexities arising from moderate levels of oncogenic replication stress (RS). While moderate levels of RS cause genome instability that contributes to OS progression, high levels of RS can lead to cell death. Notably, bromodomain and extra-terminal domain (BET) proteins (BRD2,3,4) are epigenetic readers that regulate not only transcriptional networks but also DNA replication and repair. Targeting RS through the utilization of BET inhibitors (BETi) is an underexplored treatment option in OS, and little is known about BETi-induced molecular changes and adaptive responses in OS. We hypothesize that BETi leads to decreased OS cell growth via not only remodeling of gene expression but also increased DNA damage and non-tolerated RS levels. The effect of BETi (AZD5153, BMS-986378, ZEN-3694) in clinical trials for pediatric and adult solid tumors was investigated. In vitro BETi screening of OS cell lines indicated that bivalent AZD5153 was the most potent BETi compared to monovalent BMS-986378 and ZEN-3694. However, in a longitudinal cell growth delay assay with repeat exposure of BETi at clinically relevant doses, all BETi decreased OS cell growth at similar rates. To interrogate mechanisms of action, RNA-seq analysis of BET/BRD4 inhibition via AZD5153, PROTAC (ARV-825), and BRD4 siRNA in OS cell lines revealed distinct and overlapping patterns of alterations in gene expression. A major challenge in OS therapy is mitigation of invasiveness and targeting OS metastatic foci. In all treatment groups, RNAseq analysis indicated upregulation of tumor suppressor DHRS2 an inhibitor of tumor invasion, but also TMPRSS9, a promoter of tumor invasion. This observed duality in tumor invasion mechanisms is an example of the intra-heterogeneity of the tumor response and is indicative that a second therapeutic hit is likely necessary for suppression of tumor invasiveness in remaining OS cells following BETi therapy. In vivo studies demonstrate that bivalent AZD5153 significantly suppressed tumor growth in OS patient-derived xenografts (PDXs) derived from both treatment-naïve (patient status: alive-PDX112, PDX124; deceased- PDX96, PDX115) and pre-treated metastatic (patient status: deceased-TT2/HT77) OS compared to vehicle ($p<0.05$). Therapy was well tolerated with similar drug sensitivities in female and male mice. Analysis of in-vivo PDX96 response following AZD5153 therapy indicated increased γ-H2AX and expression of pro-apoptotic genes, indicative of increased RS and cell death. At the transcript level, increased DHRS2 was evident with no changes in TMPRSS9. These data sets reveal the promise of BETi for treatment of OS PDX derived from treatment-naïve patients, some of which go on to relapse, and in OS PDX derived from metastatic sites.

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

POSTER #141

BLOOD BASED BIOMARKERS OF DNA METHYLATION ASSOCIATED WITH PLATINUM RESISTANCE IN HIGH GRADE SEROUS OVARIAN CANCER

Elnaz Abbasi Farid[6], Daniela Matei[1], Horacio Cardenas[1], Zhen Fu[2], Collin Coon[3], Adam Vieth[4], Kenneth P. Nephew[5]

[1] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University , Chicago, IL
[2] Bioinformatics and Biostatistics Core, Van Andel Institute, Grand Rapids, MI
[3] Medical Sciences, Indiana University School of Medicine, Bloomington, IN
[4] Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University , Chicago, IL
[5] Medical Sciences, Indiana University School of Medicine, Bloomington, IN, Department of Anatomy, Cell Biology and Physiology, Indiana University School of Medicine Indianapolis, IN, Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN, Bloomington, IN
[6] Medical Sciences, Indiana University School of Medicine

Email: *elabbasi@iu.edu*

High grade serous ovarian cancer (OC) is initially a responsive tumor to platinum (Pt)-based therapy. Tumor suppressor genes (TSGs) are implicated in OC initiation and progression and can be inactivated through genetic or epigenetic events. DNA methylation has been shown to silence TSGs and other cancer-related genes and is detectable in cancer cells and in blood. Here we aimed to develop a blood-based methylation assay associated with cancer and cancer recurrence in OC. We evaluated genome-wide DNA methylation in de-identified peripheral blood mononuclear cells (PBMCs) from women: without cancer (controls, n=20), newly diagnosed OC (prior to treatment, Pt-sensitive, n=50), and platinum-resistant recurrent HGSOC before and after treatment with a hypomethylating agent (HMA, Pt-resistant, n=31). The Pt-resistant patients were enrolled on NCT02901899 clinical trial testing guadecitabine and pembrolizumab. DNA extracted from PBMCs was analyzed by using Infinium MethylationEPIC BeadChips. DNA methylation analysis used R package SeSAMe to derive beta (b) values and identify differential methylated loci (DMLs) and differential methylated regions (DMRs) between groups. Bioinformatics analysis was conducted to identify biological pathways and processes, determined using Ingenuity Pathway Analysis (IPA) and KEGG, GO:BP, REAC databases. Deconvolution analyses used the HEpiDISH algorithm to determine the cellular make-up of the samples. Comparing controls vs. Pt-sensitive groups, there were 30,369 DMLs (adj. $p<0.05$, $b>10\%$), with most loci being demethylated. Enriched pathways related to presence of cancer included: mechanisms of cancer, neutrophil degranulation, and cancer-related signaling pathways (PI3K/AKT, STAT3, HGF, interleukins). The number of DMLs was greater (880, adj. $p<0.05$, $b>10\%$) in Pt-resistant vs Pt-sensitive and enriched pathways associated with Pt-resistant OC included: pathways in cancer, metabolic pathways, platelet activation, ABC transporters and signaling pathways (calcium, PI3K/AKT, MAPK, Ras, ErbB, Hippo, Wnt). Massive genome-wide hypomethylation 5 days after treatment with the HMA was seen (13,742, adj. $p<0.05$, $b>10\%$), consistent with observed LINE1 hypomethylation in PBMCs, and genome-wide hypomethylation persisted 30 days after end of HMA treatment. Enriched pathways among hypomethylated genes included: Th17 cell differentiation, virus infection, focal adhesion, signaling (chemokine, cGMP-PKG, Rap1) and hormone (thyroid, parathyroid, oxytocin, growth hormone) pathways. Deconvolution analysis identified decreased B and NK cells, monocytes, and eosinophils and a trend towards decreased $CD4^+$ and $CD8^+$ cells in Pt-sensitive OC vs controls. In contrast, neutrophils were increased in cancer vs control patients. We propose new DMLs associated with Pt-sensitive vs. Pt-resistant OC. These findings can lead to new biomarkers for HGSOC.

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

POSTER #142

DNA METHYLATION PROFILING OF PERIPHERAL BLOOD MONONUCLEAR CELLS FROM SMALL-CELL LUNG CANCER PATIENTS TREATED WITH HYPOMETHYLATING AGENT PLUS CARBOPLATIN

Elnaz Abbasi Farid[5], Zhen Fu[1], Collin M. Coon[2], Shu Zhang[2], Shadia I. Jalal[3], Kenneth P. Nephew[4]

[1] *Bioinformatics and Biostatistics Core, Van Andel Institute, Grand Rapids, MI*
[2] *Medical Sciences Program, Indiana University School of Medicine-Bloomington, Bloomington, IN*
[3] *Department of Hematology and Oncology, Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN*
[4] *Medical Sciences Program, Indiana University School of Medicine-Bloomington, Department of Anatomy, Cell Biology and Physiology, Indiana University School of Medicine, Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Bloomington, IN*
[5] *Medical Sciences Program, Indiana University School of Medicine-Bloomington*

Email: *elabbasi@iu.edu*

Small-cell lung cancer (SCLC), representing 15% of lung cancers, is one of the deadliest malignancies, with a 5-year survival rate under 7%. Despite initial sensitivity to platinum-based chemotherapy, rapid recurrence and treatment resistance remain hallmarks. Epigenetic modifications, particularly DNA methylation, play a critical role in tumor heterogeneity and resistance. Guadecitabine, a hypomethylating agent (HMA), has shown potential when combined with carboplatin. Building on these findings, we conducted a phase II single-arm trial to evaluate this combination as a second-line treatment for extensive-stage SCLC (ES-SCLC). Peripheral blood mononuclear cells (PBMCs) serve as a minimally invasive source to study systemic epigenetic changes, reflecting tumor-specific modifications. To investigate therapy-associated methylation changes, we analyzed PBMCs from 31 patients on NCT0391345clinical trial testing guadecitabine and carboplatin: 20 paired pre- and post-treatment samples, 6 pre-treatment samples, and 5 post-treatment samples. DNA was extracted using the DNeasy Blood and Tissue Kit, quantified with NanoDrop™ and Qubit spectrophotometry, bisulfite-converted, and analyzed using the Infinium HumanMethylationEPIC v2.0 platform. Data were processed with SeSAMe to generate β-value matrices. Differentially Methylated Positions (DMPs) were identified using a β-value cutoff of 0.2 and an adjusted $P < 0.05$. Functional analysis of significant DMPs was conducted via Ingenuity Pathway Analysis (IPA). Guadecitabine treatment induced 334 hypomethylated and 9 hypermethylated CpG loci, with pathway analysis revealing significant changes in SCLC and NF-kappa B signaling. Key pathways included Rho GTPase signaling (metastasis), pulmonary fibrosis signaling (lung remodeling), p75 NTR signaling (cell survival), and several other signaling pathways (ID1, MYC mediated apoptosis, immunogenic cell death). Deconvolution analysis showed decreased B cells, NK cells, monocytes, eosinophils, and a trend toward reduced CD4+ and CD8+ T cells post-treatment. Conversely, neutrophils significantly increased, suggesting an inflammatory shift in the tumor microenvironment. These findings demonstrate that guadecitabine treatment induces significant CpG methylation changes in PBMCs, impacting genes and pathways critical to cellular signaling, tissue remodeling, and survival mechanisms. The identification of key pathways, including those involved in metastasis, lung remodeling, and tumor cell survival, highlights the potential role of HMAs in modulating epigenetic and biological processes relevant to SCLC progression and therapy.

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

POSTER #143

DEEPMOP: PREDICTING HEMATOPOIETIC STEM CELL MOBILIZATION OUTCOMES USING DEEP LEARNING

Asif Adil[1,3], Stephanie Hurwitz[2]

[1] *Melvin and Bren Simon Comprehensive Cancer Center, Indiana University, Indianapolis, IN, Indianapolis, IN*
[2] *Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, Melvin and Bren Simon Comprehensive Cancer Center, Indiana University, Indianapolis, IN, Indianapolis, IN*
[3] *Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN*

Email: *asif@iu.edu*

Hematopoietic stem cell (HSC) transplantation has significantly improved survival outcomes and remains a fundamental therapy for otherwise incurable blood disorders, including hematologic malignancies.With peripheral blood now serving as the preferred source for hematopoietic stem cell collection, effective HSC mobilization is crucial for successful autologous and allogeneic stem cell transplantation. However, donor variability in $CD34^+$ cell yield after granulocyte colony-stimulating factor (G-CSF) and Plerixafor administration presents a significant challenge. Accurately predicting HSC mobilization efficiency is essential for optimizing donor selection and timing in transplantation. Current clinical prediction models rely on traditional statistical or machine learning (ML) approaches with limited predictive power.

To address this, we present DeepMOP: a *deep* learning model to *p*redict donor *mo*bilization efficiency, classifying individuals as good or poor mobilizers based on their Day 1 $CD34^+$/μL yield. Our model was trained on a cohort of 799 allogeneic donors, leveraging baseline hematological parameters, including complete blood count (CBC), age, body mass index (BMI), and other laboratory measures. We measured the performance of DeepMOP using various performance metrices including accuracy, area under the curve (AUC), and F1-score. DeepMOP outperformed the previously developed statistical and ML models, achieving the accuracy of 84.13%, AUC of 91% and F1-score of 83%. To assess model generalizability, we integrated an independent dataset of 158 donors with a reduced feature set by selecting only common variables. While this approach expanded our cohort to 957 donors, the retrained model exhibited a decline in accuracy compared to the original model, despite maintaining the same deep learning architecture. Feature importance analysis revealed that several excluded variables in the integrated dataset significantly contributed to mobilization prediction, suggesting that feature completeness is critical in addition to sample size when training predictive models for mobilization. These findings also underscore the necessity of preserving high-dimensional clinical data for robust deep learning applications in donor selection and mobilization optimization. Overall, we demonstrate the utility of a deep learning model to predict allogeneic donor mobilization success by routine clinical laboratory testing, with the goal of improving the safety, efficacy, and cost of HSC transplantation.

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

POSTER #144

MICROENVIRONMENT INDUCED ETS1-EHD1 AXIS REGULATES OVARIAN CANCER METASTASIS

Subramanyam Dasari[1,5], Ji Wang[2], Sheeza Kauser Shaik[3], Anirban K Mitra[4]

[1] *Indiana University Simon Comprehensive Cancer Center, Indianapolis, IN, Bloomington, IN*
[2] *Indiana University School of Medicine – Bloomington, Bloomington, IN, Indiana University Simon Comprehensive Cancer Center, Indianapolis, IN, Bloomington, IN*
[3] *Indiana University School of Medicine – Bloomington, Bloomington, IN, Bloomington, IN*
[4] *Indiana University School of Medicine – Bloomington, Bloomington, IN, Indiana University Simon Comprehensive Cancer Center, Indianapolis, IN, Medical and Molecular Genetics, IUSM, Indianapolis, IN, Bloomington, IN*
[5] *Indiana University School of Medicine – Bloomington, Bloomington, IN*

Email: *sudasari@iu.edu*

Ovarian cancer (OC) has the highest mortality rate among gynecologic malignancies due to widespread metastasis at diagnosis. Understanding the regulation of metastatic colonization, a critical step in metastasis, is essential for developing effective treatments. We identify key transcription factors involved in early and advanced metastasis by using an in vitro organotypic 3D omentum culture model and analyzing primary tumors and matched metastases from OC patients. Our findings reveal that reciprocal interactions between cancer cells and the metastatic microenvironment induce ETS1 expression in OC. ETS1 induction in ovarian cancer (OC) is driven by p44/42 MAP kinase signaling upon interaction with mesothelial cells at the metastatic site. Through heterotypic coculture models, conditioned medium experiments, and secretome analysis, we identified basic fibroblast growth factor (bFGF) and insulin-like growth factor-binding protein 6 (IGFBP6) were the key mesothelial factors responsible for inducing ETS1 expression in cancer cells. Treatment with recombinant human bFGF and IGFBP6 was able to replicate the effects of mesothelial cells on ETS1 expression in OC cells. Inhibition of this regulatory axis blocked the induction of ETS1 in OC cells. Single-cell RNA-seq data from high-grade serous ovarian cancer patient metastases confirmed the clinical relevance. Additionally, ETS1 regulates its target EHD1, which is crucial for metastatic colonization. EHD1 promotes ovarian cancer metastasis through migration, proliferation, and colony formation assays. It aids in recycling cell surface receptors, essential for paracrine and juxtracrine signaling with the microenvironment. As knockdown experiments showed, EHD1 also translocates Src to the cell membrane, activating FAK. Cross-linking with DSS and pulldown using biotinylated bFGF demonstrated that fibroblast growth factor receptor 1 (FGFR1) mediates the signaling cascade by binding to bFGF, which subsequently triggers ETS1 induction. We further validated the FGFR1 signaling is required for EHD1 to promote the OC metastasis through ETS1. Knockdown of EHD1 reduces, FGFR1 recycling, confirming its role in maintaining OC-microenvironment crosstalk. This study highlights ETS1's role in metastatic colonization through EHD1, revealing potential therapeutic targets to reduce metastasis and improve patient outcomes.

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

POSTER #145

PSA RESPONSE TO LU177-PSMA-617 IN PATIENTS EXPERIENCING XEROSTOMIA AND OCULAR DRYNESS IN METASTATIC CASTRATION RESISTANT PROSTATE CANCER

Rebecca Hassoun[2], Mark Tann[1], Justin Sims[1], Ashleigh Auxier[1], Sandra Althouse[1], Tareq Salous[1], Jennifer King[1], Nabil Adra[1]

[1] *Indiana University Simon Comprehensive Cancer Center, Indianapolis, IN*
[2] *Indiana University Simon Comprehensive Cancer Center*

Email: *rehass@iu.edu*

Background: Lu177-PSMA-617 (PSMA Lu177) therapy can cause dry mouth and/or eyes, as prostate specific membrane antigen (PSMA) is expressed in salivary and lacrimal glands. We evaluate the PSA response in pts with metastatic castration resistant prostate cancer (mCRPC) receiving PSMA-Lu177 who experienced dry mouth/eyes.

Methods: The Indiana University Prostate Cancer database was queried for pts with mCRPC who were treated with PSMA Lu177. Adverse Events were documented throughout the course of treatment. PSA30 and PSA50 response were analyzed among subgroups of patients who had presence/absence of xerostomia and ocular dryness. Comparisons between the groups were based on the Chi Square test, or Fisher's Exact test, if more appropriate.

Results: 144 pts with mCRPC were treated with PSMA Lu177 between November 2022 and October 2024. Included pts received at least 2 doses. Median age was 74 (53, 91). 126 pts had metastatic disease in bone, 80 in pelvic lymph nodes, 27 lungs, 20 liver, and 5 brain. 58 pts had 1 prior ARPI, 86 pts had ≥2 prior ARPI. 28 pts had no prior taxane regimen, 116 had at least 1 prior taxane. 23 pts had prior PARP inhibitor. Median follow-up from start of PSMA Lu177 was 9.77 months (1.38-26.9). 62 (43.1%) pts experienced dry mouth, 15 (10.4%) experienced dry eyes, and 13 (9%) experienced both. 92 (63.9%) pts achieved PSA30 response, and 81 (56.3%) achieved PSA 50 response.

Conclusions: Patients with mCRPC treated with PSMA Lu177 who reported dry mouth were more likely to experience PSA responses.

	Number and % of Pts Achieving PSA30 and PSA50 Response			
Characteristic (N)	PSA30 response, N (%)	PSA30 p-value	PSA50 response, N (%)	PSA50 p-value
Dry mouth · Present (N=62) · Absent (N=82)	49 (79%) 43 (52.4%)	0.0010	41 (66.1%) 40 (48.8%)	0.0377
Dry eyes · Present (N=15) · Absent (N=129)	11 (173.3%) 81 (62.8%)	0.4211	8 (53.3%) 73 (56.6%)	0.8099
Dry eyes and/or dry mouth · Present (N=64) · Absent (N=80)	51 (79.7%) 41 (51.3%)	0.0004	43 (67.2%) 38 (47.5%)	0.0180

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

POSTER #146

A SUBTYPE OF IMMUNOMODULATORY CANCER-ASSOCIATED FIBROBLASTS SHOWS PHENOTYPIC AND MOLECULAR PROFILE SIMILAR TO CYTOTOXIC T-CELL

Dharambir Kashyap[1], Jingwen Yang[1], Simpla Mahato[1], Riya Sharma[1], Malak Khalife[1], Li Zhang[1], Leng Han[1], Huda Salman[1]

[1] Brown Center for Immunotherapy, Melvin and Bren Simon Comprehensive Cancer Center, Division of Hematology and Oncology, School of Medicine, Indiana University, Indianapolis, IN 46202, USA

Email: *dbir@iu.edu*

Huda Salman[1]*(corresponsding author)

[1]Brown Center for Immunotherapy, Melvin and Bren Simon Comprehensive Cancer Center, Division of Hematology and Oncology, School of Medicine, Indiana University, Indianapolis, IN 46202, USA

Background: Cancer-associated fibroblasts (CAFs) are the most abundant cell type present in the tumor microenvironment (TME). CAFs play a crucial role in tumor progression by modulating the recruitment and activation of immune cells, including T-lymphocytes, myeloid-derived suppressor cells (MDSCs), and monocytes, leading to immunosuppression. This could be a primary reason for the inadequate response to immunotherapy recommended for highly aggressive subtypes of breast cancer. Thus, identifying the CAFs subset causing immunosuppression would lead to an improvement in existing immunotherapy.

Methods: This study included a total of 16 FFPE breast cancer samples, with four samples from each of the four subtypes. Deparaffinization was done with xylene. Tissue dissociation was done using the gentleMACS dissociator and subsequently with the manual method. FLEX-Fixed single-cell 3' gene expression strategy was used for scRNA-Seq.

Results: Using previously described gene signatures, 30 distinct clusters were categorized into various cell types, including cancer cells, fibroblasts, myofibroblasts, endothelial cells, myeloid cells, mast cells, T cells (CD4, Treg, and CD8), plasma cells, B cells, and dendritic cells. The fibroblast cluster was filtered using gene signatures such as COL1A2, COL6A1, FAP, PDPN, ACTA2, and MCAM. The resulting fibroblast cluster, consisting of 27,178 cells, was further partitioned into 13 distinct subclusters (C0-C12) based on their gene expression profiles. Gene function set enrichment analysis categorized the 13 CAFs subclusters into three major groups: i) ECM remodeling (C0, C1, and C2), which exhibited enrichment of collagen synthase enzymes, ECM proteoglycans, and cell-cell adhesion molecules; ii) Metabolism regulatory (C4 & C9-lipid metabolism, C5 carbohydrate metabolism); iii) immunomodulatory (C6, C7, C8, and C10-C12) exhibited the enrichment NF-Kß, Type-I interferon, IL-17 mediated signaling. The cluster 10, in particular, showed expression of CD3G, CD3D, CD8a, CD28, CD40L, and TRAT1, which appear to mimic the characteristics of CD8+ lymphocytes. The CAFs in the same cluster also expressed granzyme A and K, as well as PD-1. Additionally, CAF in cluster C3 exhibited NOTCH3 and Rho GTPase-mediated activity, reflecting the characteristics of stem cell types. When compared across breast cancer subtypes, the Luminal A subtype had the highest prevalence of C5, while the Luminal B subtype and TNBC showed a range of C0, C1, C2, and C10. The Luminal B subtype also exhibited another cluster, C6, with an immunomodulatory role within its stroma, whereas TNBC showed enrichment of C7. The CAFs in C7 exhibited significant expression of CD74 (CD74 molecule, a major histocompatibility complex class II invariant chain). The HER-2+ subtype, on the other hand, was associated with CAF cluster C8. The remaining clusters are distributed almost uniformly across all four subtypes.

Conclusion: The Luminal B and TNBC subtypes exhibited a high enrichment of CAF cells with an immunomodulatory role, which mimicked CD8+ lymphocytes in their stroma. The HER-2 positive subtype also exhibited CAF with immunomodulatory functions.

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

POSTER #147

UNVEILING THE DUAL NATURE OF CAR-T CELL CYTOTOXICITY: A LOOK BEYOND TARGET ANTIGENS

Malak Khalifeh[1], DHARAMBIR KASHYAP[1], Huda Salman[1]

[1] *Brown Center for Immunotherapy, Melvin and Bren Simon Comprehensive Cancer Center, Division of Hematology and Oncology, School of Medicine, Indiana University, Indianapolis, IN 46202, USA*.

Email: *mkhalife@iu.edu*

Huda Salman[1]* (Corresponsding author)

Brown Center for Immunotherapy, Melvin and Bren Simon Comprehensive Cancer Center, Division of Hematology and Oncology, School of Medicine, Indiana University, Indianapolis, IN 46202, USA.

Background: Chimeric antigen receptor (CAR) T cells are engineered to recognize specific surface antigens, distinct from the natural peptide-MHC interactions of T cell receptors (TCRs). While their precision targeting has revolutionized immunotherapy, an unanswered question remains do CAR-T cells retain endogenous TCR-mediated recognition, potentially leading to off-target effects. In this study, we investigate whether CD4-redirected CAR-T cells (CD4CAR) exhibit cytotoxicity against cells that lack the intended CAR target antigen—shedding light on their potential off-target interactions.

Methods: To explore this phenomenon, we co-cultured CD4CAR with CD4-positive KARPAS lymphoma cells, as well as two CD4-negative cancer cell lines—U87MG (malignant glioma) and SK-BR-3 (breast cancer). These interactions were compared to non-transduced CD3-positive T cells. We assessed cytotoxicity and employed single-cell RNA sequencing to dissect the molecular pathways activated during both target and off-target killing.

Results: As expected, CD4CAR-T cells exhibited potent cytotoxicity against CD4+ positive KARPAS cells. However, strikingly, both CD4CAR-T cells and intrinsic TCR-driven T cells displayed comparable cytotoxicity against CD4-negative targets, indicating an off-target effect. RNA sequencing revealed distinct molecular signatures underlying these interactions. While CD4CAR-specific cytotoxicity against KARPAS cells was mediated through TNFα-NFκB signaling, both target and off-target CAR-T cell killing involved the IL-6–JAK–STAT3 axis—a previously unrecognized pathway in CAR-T off-target activity. Target killing but not off-target involved Granzyme B.

Conclusion: Our findings reveal that beyond their intended antigen-specific activity, CAR-T cells exhibit off-target cytotoxicity driven by unique molecular pathways. Understanding these mechanisms is critical for refining CAR-T therapies, enhancing their precision, and mitigating unintended effects.

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

POSTER #148

COVALENT INHIBITION OF HPV-16 E6 RESTORES P53 AND SUPPRESSES HPV-DRIVEN TUMORIGENESIS

Lokesh Kumari[1], Anne Rietz[1], Susanna Tsueda[1], Steven Brooks[1], Essa Siddiqui, Tom Raub, Elliot Androphy[1]

[1] *Dermatology*

Email: *lkumari@iu.edu*

High-risk human papillomaviruses (HPV), particularly HPV-type 16, are major drivers of cervical, oropharyngeal, and anogenital cancers, accounting for ~5% of malignancies globally. The viral E6 oncoprotein promotes tumorigenesis by degrading p53 via the ubiquitin ligase E6AP, inhibiting apoptosis and promoting uncontrolled cell proliferation. Current treatment options, including chemotherapy, radiotherapy, and surgical resection, have limited efficacy in advanced or recurrent disease and are often associated with severe morbidities. To address this, we developed novel small-molecule E6 inhibitors that target a specific domain in HPV-16 E6, disrupting its function and restoring p53 activity.Stable p53 wild-type luciferase-expressing SiHa (HPV-16+) and RPE-1 (HPV-) cell lines were generated to monitor p53 induction. As a control, we used CRISPR-mediated mutagenesis to mutate the targeted amino acid in SiHa cell. These clones were validated via sequencing and assessed for their E6 functions in comparison to wild type SiHa cells. Screening of candidate inhibitors was followed by western blot analysis to assess p53 and p21 protein levels in HPV-16+ cervical cancer (SiHa), dysplastic (W12), and oropharyngeal cancer (UM-SCC-47, UM-SCC-104) cell lines. HPV-negative RPE-1 and SiHa E6-mutant cells served as controls. Cell viability was measured across various HPV-positive and HPV-negative epithelial cell lines using the Calcein-AM fluorescence assay. The *in-vivo* efficacy of two E6 inhibitors was evaluated using HPV-positive SiHa and UM-SCC-47, as well as HPV-negative C33a xenograft models in immunodeficient mice.HPV-16E6 inhibitors were administered daily via intraperitoneal injection.

HPV E6 inhibitors caused a dose-dependent increase in p53 and p21 protein levels (up to sixfold) in SiHa cells within 24 hours, while no such effect was observed in HPV-negative RPE-1 cells. The p53 induction was abrogated in SiHa E6-mutant cells, confirming that specific bonding to the specific amino acid in E6 is required. These E6 inhibitors selectively reduced the viability of HPV-16+ cervical (SiHa, CaSki) and oropharyngeal (UM-SCC-47, UM-SCC-104) cancer cells, with IC_{50} values of ~2-3 μM. In contrast, HPV-negative epithelial cell lines (RPE-1, HFK and NOKs) exhibited IC_{50} values >30 μM. In xenograft models, treatment with both E6 inhibitors led to significant tumor reduction (>70%) in HPV-16+ tumors, without affected tumor growth of HPV-negative C33A xenografts.Our work demonstrates that covalent inhibition of HPV-16 E6 effectively restores p53 function, leading to selective cytotoxicity of HPV-16+ cells. The profound in vivo tumor regression highlights the potential of E6 inhibitors as targeted therapeutics for HPV-driven malignancies. These findings establish E6 inhibition as a promising molecular strategy for treating HPV-16-associated cervical and oropharyngeal cancers.

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

POSTER #149

PANCREATIC CYST FLUID PROTEINS DISTINGUISH DYSPLASIA GRADE OF INTRADUCTAL PAPILLARY MUCINOUS NEOPLASM

Chunliang Liu[1], Amber Mosley[2], Ehsan Irajizad[3], Michele Yip-Schneider[4], Huangbing Wu[4], Whitney R. Smith-Kinnaman[5], Thoa Tran[4], James P. Long[3], Kim-Anh Do[3], Johannes Fahrmann[6], John M. DeWitt[7], Samir Hanash[6], C. Max Schmidt[2,4,8], Jianjun Zhang[9,4,8]

[1] *Department of Epidemiology, Indiana University Fairbanks School of Public Health*
[2] *Department of Biochemistry and Molecular Biology, Indiana University School of Medicine*
[3] *Department of Biostatistics, the University of Texas MD Anderson Cancer Center*
[4] *Departments of Surgery, Indiana University School of Medicine*
[5] *Proteomics Core, Indiana University School of Medicine*
[6] *Department of Clinical Cancer Prevention, the University of Texas MD Anderson Cancer Center*

[7] *Department of Medicine, Indiana University School of Medicine*
[8] *Indiana University Melvin and Bren Simon Comprehensive Cancer Center*
[9] *Department of Epidemiology, Indiana University Richard M. Fairbanks School of Public Health*

Email: *cl156@iu.edu*

Pancreatic cancer is the third leading cause of cancer-related death and is projected to be the second by 2030 in the US. The 5-year relative survival rate of pancreatic cancer is only 12.8% as >80% of patients have a late diagnosis. To improve survival, it is vital to detect early-stage pancreatic cancer and its precursors. One of such precursors is intraductal papillary mucinous neoplasm (IPMN), the most common type of pancreatic cysts often incidentally detected in asymptomatic patients. The current consensus guidelines largely based on imaging features have high sensitivity but low specificity in differentiating benign from malignant IPMNs, leading to widespread overtreatment of presumed "high-risk" cysts that have a low risk of malignancy on surgical pathology. Therefore, it is highly warranted to discover and validate biomarkers for improving the preoperative risk stratification of IPMN. Pancreatic cyst fluid is proximal to the tumor microenvironment and may thus harbor enhanced tumor-derived predictive biomarkers. Pancreatic cyst fluid samples were obtained from patients with pathologically confirmed low-grade (n=73) or high-grade/invasive (n=18) IPMN resected at Indiana University from 1992 to 2019. Global proteome quantitation was performed using liquid chromatography-tandem mass spectrometry. Differentially expressed proteins (DEPs) between the two groups were analyzed using LASSO regression. We discovered 152 upregulated and 74 downregulated DEPs by comparing low-grade IPMN with high-grade/invasive IPMN (all $p < 0.05$). The enriched upstream regulators of these DEPs included let-7, miR-122, IL15, and FLT1 ($p = 2.45 \times 10^{-5}$ - 5.97×10^{-3}). Five discriminatory biomarkers with the largest LASSO coefficients and each with the area under the receiver operating characteristic curve (AUCs) of >0.75 (FAHD2A, TCEAL3, TWF1, MMUT, and NTPCR) were identified. The combined five-protein model achieved an AUC of 0.96 (95% CI: 0.92, 1.00). The AUCs of cyst fluid and serum CA19-9 measured in the same patients (Spearman's rank correlation coefficient: 0.65) were 0.67 and 0.70, respectively, both of which were smaller than the AUCs of each of the top five proteins identified. Combining the five best-performing proteins with cyst fluid or serum CA19-9 yielded a near complete separation of malignant from benign IPMN (AUC: 0.981 for the cyst fluid panel and 0.997 for the serum panel). A combined analysis of TCGA and GTEx databases revealed TWF1 overexpression in pancreatic cancer ($p < 0.0001$) that was associated with poor prognosis in patients with pancreatic cancer ($p = 0.0029$). In conclusion, the present study identified a panel of cyst fluid proteins (particularly TWF1) that are predictive of malignant pancreatic cysts. If validated in other patient populations, these biomarkers could increase the accuracy of the preoperative detection of high-risk IPMN and thereby improve patient outcomes.

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

POSTER #150

CIRCULATING TUMOR DNA PROFILING REVEALS ETHNIC-SPECIFIC GENETIC ALTERATIONS IN PROSTATE CANCER

Samaneh Maleknia[4], Rebecca Hassoun[1], Nabil Adra[2], Reza Shahbazi[3]

[1] Department of Medicine/ Division of Hematology-Oncology/School of Medicine, Indianapolis, IN
[2] Department of Medicine/ Division of Hematology-Oncology/School of Medicine, Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN
[3] Department of Medicine/ Division of Hematology-Oncology/School of Medicine, Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Brown Center for Immunotherapy, Indiana University School of Medicine, Indianapolis, IN
[4] Department of Medicine/ Division of Hematology-Oncology/School of Medicine

Email: *smalekni@iu.edu*

African American men experience significantly higher prostate cancer incidence and mortality rates compared to men of other ethnic backgrounds, a disparity partially attributed to underlying biological differences. Circulating tumor DNA (ctDNA) analysis has emerged as a minimally invasive approach for clinical cancer genotyping. In this study, we utilized ctDNA profiling to investigate genetic variation in 22 African American and 66 Caucasian men diagnosed with prostate cancer. Our analysis identified 281 unique variants across 88 genes, with a higher frequency of mutations observed in African American patients. Functional enrichment through Gene Ontology and pathway analysis demonstrated strong associations between these altered genes and prostate cancer-related processes. Notably, differential engagement of genes within the KEGG prostate cancer signaling pathway suggested distinct molecular mechanisms driving disease pathogenesis between the two groups. Furthermore, we identified 25 genes potentially critical to prostate cancer development in an ethnicity-specific manner. These findings underscore the importance of incorporating ancestral genetic background into precision oncology approaches and highlight the utility of ctDNA as a tool for uncovering biologically relevant disparities in prostate cancer.

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

POSTER #151

CD24-TG2 INTERACTION DRIVES CANCER STEMNESS AND CHEMORESISTANCE IN HIGH-GRADE SEROUS OVARIAN CANCER"

Rohit Nagare[1], Fabrizio Pin[1], Ben Wamba[2], Habeebunnisa Begum[1], Tushir Singh[2], Salvatore Condello[1]

[1] School of Medicine, Indiana University
[2] University of California

Email: *rpnagare@iu.edu*

Ovarian cancer (OC) remains a major clinical challenge due to its high mortality, frequent relapse, and resistance to therapy. Tissue transglutaminase 2 (TG2), involved in protein cross-linking, extracellular matrix (ECM) remodeling, and signaling, is linked to OC, OCSC maintenance, and chemo-resistance mechanisms. TG2 has a fibronectin-binding domain within its N terminus. The complex between TG2 and fibronectin (FN) is stabilized by direct interactions with integrins and ECM.

This study aims to investigate the role of CD24 and TG2 interaction in high-grade serous ovarian cancer (HGSOC). The association between the two proteins was assessed using a TG2-blocking peptide (BP), alone and in combination with a CD24-targeting antibody (CD24-BP). The impact of these treatments on CSC-related pathways was evaluated through expression of OCSCs markers (Nanog, OCT-4, SOX2, ALDH1A1) and spheroid formation using OC cell lines and primary tumors. In vivo tumor models were used to evaluate the effects of BP and CD24-BP on tumor volume. The expression of two proteins were evaluated across the panel of cell lines and primary samples. The physical interaction two proteins and its associated protein complexes was investigated by co-immunoprecipitation (Co-IP) using SKOV3 and OVCAR4 ovarian cancer cell lysates. Co-existence CD24 and TG2 was confirmed by fractionation of lipid rafts followed by western blotting using OVCAR4 cell line lysate.

Treatment with either BP or CD24-BP showed reduced expression of CSCs specific markers Nanog, OCT-4, SOX2, ALDH1A1 and reduced spheroid size in both OC cell lines and patient-derived spheroids, indicating impaired self-renewal potential. Further, in vivo studies demonstrated that BP or CD24-BP treatment reduced the tumor volume and size as compared controls. Among the cell lines, OVCAR4, OVCAR5 and SKOV3 showed higher expression of TG2 while CD24 was expressed in all cell lines. TG2 and CD24 expression was higher in chemo resistant samples as compared to chemo naive. The Co-IP demonstrated that TG2 specifically co-precipitated with CD24, and vice versa, confirming their physical interaction. Lipid raft fractionation revealed that both TG2 and CD24 co-localize specifically within lipid raft domains, suggesting that CD24 may facilitate the membrane localization and functional enhancement of TG2 under chemotherapy-induced stress.

These findings support the hypothesis that CD24 and TG2 form a regulatory axis that contributes to tumor progression, chemoresistance, and CSC maintenance in ovarian cancer. Understanding of TG2-CD24 interaction opens new strategies for therapeutic intervention as both TG2 and CD24 are potential biomarkers. Future research involves exploration of mechanism behind this interaction and exploit it for developing new therapeutic strategies.

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

POSTER #152

CRISPR NANOFORMULATIONS TARGETING CASTRATION-RESISTANT PROSTATE CANCER

Noah Richardson[1,2], Sanam Rezaei Benam[1], Samaneh Maleknia[1], Reza Shahbazi[1,3,2,4]

[1] Division of Hematology/Oncology, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN
[2] Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN
[3] Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN
[4] Brown Center for Immunotherapy, Indiana University School of Medicine, Indianapolis, IN

Email: *noaricha@iu.edu*

Advanced metastatic prostate cancer remains an incurable disease and novel therapies are greatly needed to improve outcomes. Initially, metastatic prostate cancer responds to androgen deprivation therapy (ADT) blocking the androgen receptor (AR) signaling pathway responsible for growth and proliferation. Eventually, resistance develops and although circulating

androgens remain reduced,signaling through the AR pathway continues through alternative mechanisms. This stage, known as castrate-resistant prostate cancer (CRPC), is characterized by relentless disease progression despite ongoing ADT. Changes in the AR signaling axis, such as receptor duplications, constitutively active splice variants, and point mutations, confer resistance to ADT by circumventing systemic androgen depletion. Furthermore, intratumoral androgen synthesis and receptor responsiveness to other steroid ligands contribute to the persistent activity of the AR. The continued biological significance and reliance of AR signaling in CRPC has made the definitive knockout (KO) of the gene an enticing target.

We have used antibody-coated lipid nanoparticles (LNP) to specifically direct CRISPR nanoformulations to prostate-specific membrane antigen (PSMA) positive CRPC cells and knock out the AR. Our CRISPR formulation (PSMA-LNP) has increased safety and specificity with both extracellular and intracellular prerequisites for CRISPR activity. Intracellular activation of the CRISPR sgRNA nuclease complex will be mediated by sensing cell-specific miRNA uniquely identified in malignant cells. We have screened miRNA databases and a CRPC cell line C4-2B identifying candidate miRNA not expressed significantly in other healthy tissues. Using miRNA-flanked sgRNA CRISPR formulations our system once shuttled to PSMA expressing C4-2B cells allows assembly of Cas9 protein and sgRNA in a cell-specific manner leading to AR knock out and cell death. Starting with identifying a standard design AR sgRNA guide, our western blot results show 50-80% AR knockout with60-90% gene editing efficiency analyzed by T7EI, and TIDE analysis. Our time-lapse Incucyte live cell analysis shows preferential targeting of PSMA-LNP to C4-2B cell lines in comparison to control malignant and primary cell lines *in vitro*. Delivery of our PSMA-LNP formulation in a mouse orthotopic model of CRPC shows preferential delivery to CRPC tumors.

Currently, we are continuing our studies selecting miRNA-responsive flanked-sgRNA guides and optimizing construct. Our findings demonstratethe feasibility of targeted CRISPR gene editing to treat prostate cancer and overcome known resistance mechanisms of AR-based therapeutics with improved safety features.

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

POSTER #153

THE IMPACT OF SOCIAL DETERMINANTS OF HEALTH ARE GREATER IN PATIENTS WITH OSTEOSARCOMA THEN EWING SARCOMA

Spencer Richardson[1], Ben Johnson[3], Ateik Almalahi[3], L. Daniel Wurtz[1], Devin Conway[1], Christopher Collier[1]

[1] *Department of Orthopaedic Surgery, Indiana University School of Medicine, Indiana University Health, Indianapolis, IN*
[3] *Indiana University School of Medicine, Indiana University Health, Indianapolis, IN*

Email: *spenrich@iu.edu*

Background: We and others have previously reported the association of patient demographic factors with survival and treatment response in primary bone sarcoma in large database studies. However, these studies are limited by data availability to further investigate associated factors contributing to these disparities. The objective of this study was to identify whether available demographic factors are associated with treatment and outcomes in patients with pediatric bone sarcoma and explore contributing factors at a single institution.

Methods: An institutional database identified 71 patients with osteosarcoma and 46 patients with osseous Ewing sarcoma diagnosed between 2010-2023. Demographic data including the Area Deprivation Index (ADI) and treatment outcomes were collected from patient records. Patients were stratified by socioeconomic status using 75th percentile and greater representing increased social disadvantage. Survival was compared using Log-rank Test.

Results: Low utilization of outpatient clinics was found in both Ewing sarcoma (46%) and osteosarcoma (38%) cohorts with high ADI deprivation. Most patients with high ADI deprivation were initially evalauted in the emergency department. Patients with osteosarcoma and high ADI scores had longer median symptoms duration prior to diagnosis compared to low deprivation scores [13.1 weeks (IQR: 6.7-19.2) vs 6.7 weeks (IQR:4.0-11.3), $p<0.01$]. There was no difference in symptoms duration between ADI groups in patients with Ewing Sarcoma ($p=0.39$). High ADI deprivation patients with osteosarcoma needed more additional weeks to complete planned chemotherapy [7.9 (IQR: 6.3 to 11.7) versus the low ADI deprivation group [5.6 weeks (IQR: 3.9 to 9.0) ($p=0.012$)]. Overall survival and metastasis free survival were lower in osteosarcoma patients with ADI > 75th percentile but no difference was seen in patients with Ewing sarcoma.

Conclusions: Measures of socioeconomic disadvantage are associated with presentation, treatment, and survival in pediatric bone sarcoma. Further work is needed to identify if prolonged diagnostic time and treatment interruptions drive the differential outcomes in osteosarcoma

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

POSTER #154

ENHANCING TH9 CELL EFFECTOR FUNCTIONS THROUGH PTPN2 BLOCKADE

Jiazhi Xu[4], Chiranjeevi Tikka[1], Linlin Guo[2], Kent Williams[3], Souleymane Abdoul-Azize[2], Sophia Shelburn[2], Jasmine Abulail[2], Reza Shahbazi[2], Lionel Apetoh[3]

[1] Department of Medicine, Division of Hematology/Oncology, Indiana University School of Medicine, Indianapolis, IN, Indianapolis, IN

[2] Department of Microbiology & Immunology, Indiana University School of Medicine, Indianapolis, IN, Indianapolis, IN

[3] Department of Microbiology & Immunology, Indiana University School of Medicine, Indianapolis, IN, Brown Center for Immunotherapy Immune Monitoring Core, Indiana University School of Medicine, Indianapolis, IN, Indianapolis, IN

[4] Department of Microbiology & Immunology, Indiana University School of Medicine, Indianapolis, IN

Email: *xu19@iu.edu*

The adoptive transfer of T cells to patients suffering from advanced skin cancer has led to complete responses. However, a large fraction of patients bearing solid tumors fail to respond to this treatment due to poor T cell persistence in the tumor microenvironment. Therefore, an improved knowledge of T cell biology is required to enhance their in vivo clinical efficacy. IL-9-secreting CD4 T (T_H9) cells are a novel CD4 T cell subset that features an exceptional ability to treat melanoma-bearing mice in vivo over T_H1 and T_H17 cells, attributed to their resistance to dysfunction, apoptosis, and their ability to self-renew in vivo. The cellular events controlling to T_H9 cell differentiation remain incompletely understood. Our results reveal that the disruption of protein tyrosine phosphatase non-receptor type 2 (PTPN2) signaling using an orally bioavailable inhibitor, ABBV-CLS-484, enhances T_H9 cell differentiation and effector functions. Pharmacological blockade and knock down of PTPN2 both increased T_H9 cell polyfunctionality, including higher secretion of IL-9 as well as granzyme B. Mechanistic investigations reveal that PTPN2 inhibition promotes enhanced phosphorylation of the STAT5 transcription factor, leading to increased IL-9 secretion. Adoptive transfer of PTPN2 inhibitor-treated T_H9 cells into B16-OVA melanoma bearing mice exhibited improved anti-cancer efficacy compared to both untreated T_H9 cells and control groups. Mechanistic studies showed that the adoptive transfer of PTPN2 inhibitor-treated T_H9 cells resulted in increased CD8+ T cells recruitment to the tumor microenvironment, along with enhanced IFN-γ secretion by CD8+ T cells. Our findings strongly suggest that PTPN2 blockade can potentiate the anti-cancer functions of T_H9 cells in vivo and provide compelling evidence for the potential of this approach in improving adoptive cell therapies for cancer treatment.

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

POSTER #155

EX VIVO NEURONAL ACTIVITY OF PERIPHERAL SENSORY NEURONS DERIVED FROM PATIENTS WITH TAXANE-INDUCED PERIPHERAL NEUROPATHY

Erica Cantor[1,3], Fei Shen[2], Santosh Philips[2], Guanglong Jiang[1], Bryan Schneider[2]

[1] *Medical & Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN*
[2] *Hematology/Oncology, Indiana University School of Medicine, Indianapolis, IN*
[3] *Hematology/Oncology, Indiana University School of Medicine*

Email: *ericant@iu.edu*

Peripheral neuropathy is a common and potentially devastating side effect of taxanes, a widely used anticancer therapy. Taxane-induced peripheral neuropathy (TIPN) can lead to dose reductions and cessation of treatment and negatively impacts patient quality of life. We have successfully utilized a previously established human induced pluripotent stem cell (iPSC)-derived peripheral sensory neuron (iPSC-dSN) model to study neuronal activity *ex vivo* using calcium imaging. In this study, we utilized this model to examine the association of clinical neuropathy status with peak intracellular calcium response to capsaicin.

Twenty-six iPSC lines were reprogrammed from the peripheral blood of patients from phase II clinical trial EAZ171 and were subsequently differentiated into iPSC-dSNs. Cell lines were derived from patients who either did not experience TIPN (controls) or did experience TIPN (cases) after receiving taxane treatment in the study. After four weeks of maturation, iPSC-dSNs were pre-treated for 48 hours with either vehicle (DMSO) or a taxane (10 nM docetaxel or 100 nM paclitaxel). Live-cell fluorescence-based calcium imaging was subsequently conducted by capturing one minute of baseline recording followed by the addition of either KCl (control) or capsaicin (agonist), after which the response was recorded for an additional minute. Data were normalized to the average baseline fluorescence and peak intracellular response was determined as the maximum fluorescence detected during the response period. Peak responses of iPSC-dSNs were compared between non-TIPN controls and TIPN cases.

Overall, taxane treatment resulted in a significant reduction in peak intracellular calcium response regardless of agonist or neuropathy status (p-value = 4.55×10^{-17}). The magnitude of this change was not significantly different in response to KCl depolarization between non-TIPN controls and cases. However, there was significantly less reduction in peak response to capsaicin in taxane pre-treated cells from TIPN cases (p-value = 0.0396). The overall peak response was significantly lower in vehicle pre-treated cells derived from cases than those from controls (p-value = 0.00436), suggesting a baseline difference in neuronal activity between non-TIPN controls and TIPN cases.

The results of this study indicate that cells derived from TIPN patients had a lower innate response to capsaicin, an activator of TRPV1. These results may suggest inherent differences in the neuronal function of cells derived from patients who experience TIPN compared to those who do not. These studies may lead to insights into the mechanism of TIPN and help to develop potential targets for treatment.

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

POSTER #156

CEREBRAL SPINAL FLUID (CSF) PREDICTORS OF DEVELOPING DISSEMINATED NECROTIZING LEUKOENCEPHALOPATHY IN LEPTOMENINGEAL CARCINOMATOSIS (LMC): A SINGLE INSTITUTIONAL REVIEW

Ucheoma Eze

Email: *uceze@iuhealth.org*

BACKGROUND: LMC is a rapidly fatal rare complication of systemic cancer with metastasis to the leptomeninges. Despite improved diagnostic capabilities, treatment options remain few and most clinical trials exclude LMC patient participation. Whole brain radiation therapy (WBRT) and intrathecal (IT) chemotherapy are commonly employed and there is growing evidence for systemic therapies that cross the blood brain barrier. However, complications related to treatment can confound response assessment, leading to early discontinuation of effective therapies. One such poorly understood complication is the development of disseminated necrotizing leukoencephalopathy (DNL). DNL is a, typically self-limited, rare

treatment-related radiographic finding variably associated with clinical decline and often misdiagnosed as progression or infection. DNL has been reported in patients exposed to IT chemotherapy and CNS irradiation. Our lab published improved LMC control with extended survival after standardization of Ommaya clinic IT treatment regimens, although with increased incidence of DNL.

METHODS: We retrospectively analyzed patients with LMC treated with IT chemotherapy after WBRT between 2017–2020. We reviewed sequential brain imaging over the treatment course to determine if there were predictive CSF markers associated with the development of DNL.

RESULTS: Of 41 patients evaluated, 24% (n=10) developed DNL at a median 24-weeks after start of IT chemotherapy and WBRT. Of the patients with radiographically assessed DNL, 80% (n=8) experienced elevated CSF protein and 40% (n=4) had elevated white blood cells within 8-weeks prior to radiographic determination of DNL. Cytology remained benign and glucose was not significantly changed prior to DNL development.

CONCLUSION: Given the poor outcome of LMC, there is a need for treatment standardization and better understanding of treatment-associated radiographic changes. Evaluation of CSF markers to predict impending development of DNL in this treatment population might limit radiographic misdiagnoses and patient clinical decline as well as offer a potential window of opportunity for treatment de-escalation.

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

POSTER #157

EXPLORING THE IMPACT OF OXYGEN-DEPENDENT ERK/PI3K/AKT SIGNALING IN MALIGNANT GLIOMAS: IMPLICATIONS FOR TUMOR PROGRESSION AND THERAPEUTIC RESISTANCE

Natasha Hockaden[1,3], Elise O'Herron[2], Angela Richardson[2]

[1] *Indiana University School of Medicine, Indianapolis, IN, Indianapolis, IN*
[2] *Department of Neurological Surgery, Indiana University School of Medicine, Indianapolis, IN, Indianapolis, IN*
[3] *Department of Neurological Surgery*

Email: *nhockade@iu.edu*

Glioblastoma (GBM) is a highly aggressive brain tumor with a median survival of just 15-20 months, despite treatments like surgical resection, temozolomide chemotherapy, radiation, and tumor-treating fields, which offer only a modest survival benefit of about 3 months. Recurrence is nearly inevitable, with survival rates of 17% at 2 years and 10% at 5 years. Immunotherapy has shown limited success, primarily due to the immunosuppressive tumor microenvironment created by glioma stem cells (GSC), which prevent effective immune responses against the tumor. Among the factors influencing GBM progression, oxygen levels play a crucial role in modulating cellular behavior, metabolism, and therapeutic response. ERK signaling plays a key role in cancer cell survival and therapy resistance, with its activity influenced by oxygen levels in the tumor microenvironment. In normoxic conditions, ERK is activated through receptor tyrosine kinases and AKT, promoting tumor growth and resistance. In physoxic conditions, low oxygen alters ERK signaling, contributing to resistance through crosstalk with the PI3K/AKT pathway. This adaptive response is further shaped by fluctuating oxygen levels, affecting cancer cell reprogramming. Understanding these oxygen-dependent changes in ERK signaling is crucial for improving cancer therapy outcomes. This study investigates the differential effects of normoxia and physoxia on patient derived malignant glioma cells, specifically focusing on ERK and PI3K/AKT signaling, cellular proliferation, survival, changes in cancer phenotype, and gene expression changes. Previous studies have led us to investigate the effect of lower oxygen levels on cancer phenotypes and the ERK/PI3K/AKT signaling pathways. These findings will further emphasize the importance of the tumor microenvironment in influencing glioblastoma biology and the development of effective therapies.

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

POSTER #158

INDIANA UNIVERSITY GENOME EDITING CENTER

Ankeeta Koirala[5], Kyle O'Connor [1], Samantha Bare[1], Max Toubin[1], Anastasiya Vydra[1], Destinee Thomas[1], Charles Mullighan[2], Peter Murray[3], Joshua Huot[4], Hanying Chen[1], Stephane Pelletier[1]

[1] Indiana University Genome Editing Center, Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN

[2] Department of Pathology, St. Jude Children's Research Hospital , Memphis, TN

[3] Max Planck Institute of Biochemistry

[4] Department of Anatomy, Cell Biology and Physiology, Indiana University School of Medicine, Indianapolis, IN

[5] Indiana University Genome Editing Center, Department of Medical and Molecular Genetics, Indiana University School of Medicine

Email: *akoiral@iu.edu*

The Indiana University Genome Editing Center (IUGEC), established in July 2020, is an IUSM- and CTSI-designated shared resource whose mission is to provide both Indiana University and external investigators access to state-of-the-art genome editing technologies by assisting with the generation of genetically modified organisms in a time-effective manner. The IUGEC utilizes various technologies including CRISPR-based genome editing systems, conventional gene targeting, and transgenesis to engineer genetically modified transformed cell lines, stem cells, and animal models. These technologies will help identify and validate novel therapeutic targets, accelerate translational research, and advance precision medicine. Our group can assist with engineering various types of mutations relevant to cancer biology including tumor promoting missense mutations, chromosomal translocations, conditional translocations, and others, both in vivo and in vitro.

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

POSTER #159

DISCOVERY, DEVELOPMENT, AND CHARACTERIZATION OF NOVEL TIGIT B-CELL EPITOPE PEPTIDE VACCINES SHOW ANTI-TUMOR IMMUNITY IN MULTIPLE SYNGENEIC MICE MODELS

Jay Overholser[3], Guo Linlin[1], Pravin Kaumaya[2]

[1] *Department Microbiology and Immunology, Indianapolis, IN*
[2] *Arthur G. James Cancer Hospital/Comprehensive Cancer Center. Department Microbiology and Immunology., Indianapolis, IN*
[3] *Arthur G. James Cancer Hospital/Comprehensive Cancer Center*

Email: *jpoverho@iu.edu*

Cancer immunotherapy with checkpoint inhibitors (i.e. targeting PD-1,PD-L1, and CTLA-4 have led great clinical benefits for decades in multiple type of cancers. However, treatment resistance, relatively low percentage of patients' fully responding, and severe monoclonal antibody associated side effects urgently need to be resolved. In the current study, we identified, designed and synthesized several new B-cell epitopes peptide vaccines targeting T-cell immunoglobulin and ITIM domain (TIGIT). Immunization with chimeric TIGIT -B-cell epitopes with promiscuous T cell epitopes MVF- or TT3- elicited high titers of polyclonal antibodies that show high affinity to recombinant human TIGIT protein. In the syngeneic mouse tumor models, the MVF-TIGIT-2 (131-148) and MVF-TIGIT-4 (111-129) were more efficacious inhibiting tumor growth and prolonged mice survival rates overall other candidates especially in the BALB/c mammary tumor model and melanoma model of B16-F10 on C57BL/6J mouse. Detailed results of the best TIGIT epitopes will be presented.

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

POSTER #160

WHAT I WISH I KNEW ABOUT THE SURGICAL OPTIONS FOR BONE CANCER: "FUNCTION IS MORE IMPORTANT THAN LOOKS!"

Janet Panoch[3], Clayton Hicks[1], Christopher Collier[2]

[1] *IU School of Medicine, Indianapolis, IN*
[2] *Department of Orthopaedic Surgery, IU School of Medicine, Indianapolis, IN*
[3] *Walther Supportive Oncology Program, General Internal Medicine, IU School of Medicine*

Email: *jpanoch@iu.edu*

Background. Osteosarcoma and Ewing sarcoma rare, primarily pediatric bone cancers with about 1200 cases annually in the United States. Most tumors are in the lower extremity with surgical options that may include amputation, limb salvage, or rotationplasty. Families and patients may be engaged in shared decision making with their surgeon since the oncological outcome is the same. Little is known about the patient's perspective of life after surgery.

Methods. Eligible participants were over age 18, diagnosed with osteosarcoma or Ewing sarcoma or be a caregiver/family member. A Survey Monkey survey was disseminated to four Osteosarcoma/Ewing sarcoma Facebook support groups with three questions that included When you think about life after surgery, what do you wish you knew? Responses were deidentified and analyzed by two researchers to explore life after surgery for people with bone cancer.

Results. After four weeks, there were 155 responses, mostly parents (n=99). The majority of participants were under the age of 18 when they had their first surgery (n=123) with a tumor in the lower limb starting at the femur (n=134). More than half of the participants (n=86) indicated that their first surgery was limb salvage. Time since the first surgery ranged from two weeks to 40 years.

For participants who had limb salvage, nearly all wish they knew about the functional limitations. They also wish they knew about common issues (infection, implant loosening, and chronic pain) and the need for additional surgeries. They wish they knew how long the limb salvage implant would last and that each surgery had "diminishing returns" for success. Some participants chose to have an amputation or rotationplasty after limb salvage because of these issues.

For those who had amputation or rotationplasty, they wish they knew about the cost and process of getting fitted for prosthetics, especially for a growing child.

For all participants, they wish options were explained better with more focus on function. Regrets were about having a surgery that did not give them a good quality of life.

I wish I knew about the limited mobility I would have. I wish I knew that amputation may be a benefit and not a loss. I wish I would've known that I would be electing for amputation at 30 years old. I wish I knew that "at least I still have my leg" isn't the win against cancer that I wanted. – patient who had limb salvage at age 14

Conclusion. These findings suggest that patients and families feel unprepared for life after surgery. They regret not having options or not being informed about the limitations of options offered to them. Patient education is needed for making this pivotal surgical decision.

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

POSTER #161

Remove

MODELING ACQUIRED RESISTANCE TO PARP INHIBITION IN METASTATIC CASTRATION-RESISTANT PROSTATE CANCER

Erin Perkins[2], Jennifer Rooks[2], Asmaa El-Kenawi[1,2]

[1] *Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN*
[2] *Department of Urology, IU School of Medicine*

Email: *erinperk@iu.edu*

Although anti-cancer treatments often show initial efficacy, many tumors ultimately develop resistance, resulting in therapeutic failure and cancer recurrence. To investigate the underlying mechanisms of acquired resistance, in vitro models are essential, as they enable detailed analysis of changes in signaling cascades, transcriptional activity, epigenetic regulation, and cellular metabolism associated with reduced drug sensitivity. Poly (ADP-ribose) polymerase inhibitors (PARPi), such as Olaparib and Rucaparib, are used in the treatment of metastatic castration-resistant prostate cancer (mCRPC). These agents disrupt DNA repair pathways, leading to growth arrest and cell death, particularly in the context of DNA repair deficiencies or when combined with other therapies. To study metabolic mechanisms of resistance, we established a protocol for generating PARPi-resistant prostate cancer cell lines. DU-145 cells were plated in 96-well format and exposed to varying concentrations of Olaparib to determine the IC75, which was subsequently used to select for resistant populations. Cells were then seeded in 6-well plates and continuously cultured, with passaging upon confluency, to promote the emergence of resistant clones. Given that prolonged PARPi exposure can lead to resistance, the development of these resistant cell lines provides a valuable model for understanding resistance in mCRPC and optimizing therapeutic strategies.

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

POSTER #162

SINGLE NUCLEUS CHROMATIN ACCESSIBILITY AND TRANSCRIPTOME ANALYSES REVEAL ABERRANT INTER-CELLULAR COMMUNICATION IN INFLAMMATORY BREAST CANCER

Poornima Bhat-Nakshatri[3], Cihat Erdogan[1], Hongyu Gao, Yunlong Liu, Harikrishna Nakshatri[2]

[1] *Molecular Genetics, Indianapolis, IN*
[2] *Indiana University School of Medicine, IN 46202, USA Richard L Roudebush VA Medical Center, Indianapolis, IN 46202, USA, Indianapolis, IN*
[3] *Surgery*

Email: *pnakshat@iu.edu*

International efforts focused on discovery of biological targets in inflammatory breast cancer (IBC) have suggested that aberrant inter-cellular relationship instead of genomic aberrations is the key driver of IBC. To identify such IBC-specific aberrations, we generated single nucleus chromatin accessibility and transcriptome atlas of IBCs (9,628 nuclei from three donors) and compared IBC atlas with a similar atlas of the healthy breast (81,735 nuclei from 92 donors). We recently reported single nucleus atlas of breast tissues of healthy women of diverse genetic ancestry (Nature Medicine in press). In that report, we described markers that identify three major epithelial cell subtypes [Basal-myoepithelial (BM), luminal adaptive secretory precursor (LASP), luminal hormone sensing (LHS)], two endothelial cell subtypes, two adipocyte subtypes, fibroblasts, macrophages, and T cells of the healthy breast. We also showed that LHS cells are the likely cells-of-origin of Luminal A, Luminal B and HER2+ breast cancers. Unlike these breast cancer subtypes, IBC does not appear to have a cell-of-origin, as epithelial cells in IBCs shared gene expression pattern across BM, LASP and LHS cells. Similar results were obtained when comparison was restricted to ancestry-specific healthy breast atlas. However, individual gene level expression differences affecting specific signaling pathways were observed in epithelial cells of IBCs. LASP, and LHS cells of IBCs overexpressed GPR137C, a positive regulator of mTORC1 signaling, and HS6ST3, an enzyme required for heparan sulfate synthesis. LASP and LHS cells of IBCs displayed activation of mitotic and matrix metalloproteinase signaling, respectively. Endothelial cells of IBCs, which showed significant gene expression differences compared to endothelial cells of the healthy breast, displayed enhanced integrin cell surface interaction but loss of cell junction organization. Most critically, BM, LASP, LHS, and endothelial cells of IBCs compared to their counterparts in the healthy breast showed downregulation of TEX14, an inter-cellular bridge forming factor in germ cells and a regulator of mitosis. Downregulation of TEX14 expression in these cells was accompanied with changes in the chromatin accessibility patterns of this gene. These results reinforce the notion that defective inter-cellular communication is a hallmark of IBC and TEX14 expression levels may serve as a biomarker of this defect. We suggest that greater attention to vascular biology and vascular-epithelial cell communication has to be given for better understanding of IBC biology and therapeutic targeting.

Translational/Clinical Research ***Research specialist***

POSTER #163

ADJUVANT CHEMOTHERAPY FOR INADEQUATE LYMPHADENECTOMY IN STAGE II SMALL BOWEL ADENOCARCINOMA

Jackson Baril[1], Karl Bilimoria[1], Eugene Ceppa[1], Michael House[1], Anita Turk[2], Thomas Maatman[1], Alexandra Roch[1], C. Max Schmidt[1], Ryan Ellis[1]

[1] *Department of Surgery, Division of Surgical Oncology, Indiana School of Medicine*
[2] *Department of Medicine, Division of Medical Oncology, Indiana School of Medicine*

Email: *jbaril@iu.edu*

Introduction: Adjuvant chemotherapy (AC) is considered for patients with stage II small bowel adenocarcinoma (SBA) with an inadequate lymphadenectomy; however, the prognostic role of additional high-risk features (T4 primary, positive resection margin, poorly differentiated tumor, or lymphovascular invasion) to further stratify patients with an inadequate lymphadenectomy is unknown. The objectives of this study were to (1) describe the utilization of AC after inadequate lymphadenectomy for patients with stage II SBA, (2) identify factors associated with receipt of AC, and (3) examine the association between AC and survival for patients with or without additional high-risk oncologic features.

Methods: Patients with stage II SBA were identified using the National Cancer Database between Jan 1, 2004, and Dec 31, 2021. Factors associated with receipt of AC were assessed using multivariable logistic regression. Survival was assessed by Kaplan-Meier method and Cox proportional hazards.

Results: Overall, 1,765 patients (mean age 68 years; 815 (46.2%) female) with stage II SBA had an inadequate lymphadenectomy. Of those patients, 1,013 (57.4%) had at least one additional high-risk feature and 525 (29.8%) received AC. Several clinicopathologic features were associated with receiving AC including T4 primary tumor (vs. T3 primary tumor, adjusted odds ratio (aOR) 1.97; 95%CI 1.50-2.57; p<0.001), poor grade tumor (vs. moderate, aOR 1.42, 95%CI 1.07-1.89; p=0.015), and positive resection margin (vs. negative margin, aOR 1.62, 95%CI 1.12-2.35; p=0.011). Receipt of AC, compared with no receipt of AC, was associated with improved 5-year overall survival (54.2% vs 44.5%; Hazard Ratio for mortality (HR) 0.78, 95%CI 0.66-0.94). When stratified by the presence of additional high-risk features, receipt of AC, compared with no receipt of AC, was associated with improved 5-year survival in patients with additional high-risk features (49.3% vs 34.2%; HR 0.73, 95%CI 0.59-0.90) but not in patients without additional high-risk features (65.4% vs 55.2%; HR 0.91, 95%CI 0.64-1.28).

Conclusion: Among patients with stage II SBA, receipt of AC was associated with improved survival in patients with inadequate lymphadenectomy and any additional high-risk feature. High-risk pathologi features pay assist in recommending AC, and multiple patient and pathologic variables should be considered in decisions regarding AC.

Translational/Clinical Research ***Resident***

POSTER #164

MANAGEMENT OF PROGRESSIVE BRAIN METASTASES IN PATIENTS (PTS) WITH RELAPSED GERM-CELL TUMOR (GCT) TREATED WITH SALVAGE HIGH-DOSE CHEMOTHERAPY (HDCT)

Virginia Olivier, MD, Jennifer King, MD, Rafat Abonour, MD, Rebecca Hassoun, MD, Sandra Althouse, MS, Tareq Salous, MD , Nasser Hanna, MD, Lawrence Einhorn, MD, Nabil Adra, MD

Email: *violiv@iu.edu*

Background:

Previous data confirm that pts with relapsed GCT and progressive brain metastases (mets) can be cured with HDCT and peripheral blood stem cell transplantation (PBSCT)1. Still, there is uncertainty with the optimal management sequence of these pts prior to transplant. Here, we describe the management and outcomes of a larger cohort of these pts.

Methods:

The prospectively maintained Indiana University testicular cancer database was queried for pts with relapsed metastatic GCT who were to undergo HDCT with PBSCT and were noted to have progressive brain mets at time of relapse. Baseline characteristics were summarized. The Kaplan-Meier method was used to analyze progression free survival (PFS) and overall survival (OS).

Results:

49 pts met eligibility. Median age was 28.7yrs (16.6-51.5). Primary site was testis in 44 (90.0%), mediastinal in 4 (8.2%), and retroperitoneum in 1 (2.0%). All patients had nonseminomatous disease. Primary tumor predominant histology was choriocarcinoma (34.7%), embryonal (32.7%), mixed (18.4%), teratoma (6.1%), yolk sac tumor (4.1%), and seminoma (2.0%). IGCCCG risk at diagnosis was poor in 91.8%, intermediate in 2.1%, and good in 6.1%. 24 pts (49%) were platinum refractory at HDCT. 17 pts (34.7%) had brain mets at diagnosis; the rest developed at relapse.

26 pts (53.1%) went straight to HDCT without localized treatment to progressive brain mets. 8 (16.3%) underwent craniotomy and 8 (16.3%) had radiation prior to HDCT. 3 of the pts who had radiation had stereotactic radiosurgery (SRS); 5 had whole brain radiotherapy (WBRT). 7 pts (14.3%) underwent craniotomy then radiation prior to HDCT. 5 pts (15.2%) were symptomatic from progressing brain mets at HDCT. 1 of these had mild visual symptoms and went straight to HDCT. 1 pt began having headaches the day before HDCT and given proximity to initiation, HDCT was continued. The other 3 symptomatic pts had previously failed localized treatment for brain mets. 22 pts (44.9%) progressed after HDCT. At a median follow-up of 3.8 yrs (0.6-14.8), 16 pts (32.7%) were alive with no evidence of disease, 10 (20.4%) were alive with disease, 20 (40.8%) died of disease, and 3 (6.1%) died of other causes. 2-yr PFS was 43.9%; 2-yr OS was 73.2%.

Conclusions:

Pts with relapsed GCT with progressive brain mets can be cured with HDCT with PBSCT. Management of progressive brain mets should be individualized for each pt, taking into account extent of brain mets and presence of symptoms.

1Kalra M, et al. Cancer. 2020; 126(6): 1202-1207.

Translational/Clinical Research ***Resident***

POSTER #165

MANAGEMENT OF RELAPSED PRIMARY RETROPERITONEAL (RP) GERM-CELL TUMOR (GCT) AFTER FRONT-LINE CHEMOTHERAPY

Kirsten Lewis[2], Rebecca Hassoun[1], Sandra Althouse[1], Tareq Salous[1], Nasser Hanna[1], Lawrence Einhorn[1], Nabil Adra[1], Jennifer King[1]

[1] *Indiana University School of Medicine, Indianapolis, IN*
[2] *Indiana University School of Medicine*

Email: *kirlewis@iu.edu*

Background

Primary RP GCT represents a rare subset of extragonadal GCTs. While front-line therapy remains similar to gonadal GCT, there is limited data on management of relapsed disease for these patients (pts). Here, we describe management and outcomes of pts with relapsed primary RP GCT.

Methods

The prospectively maintained Indiana University testicular cancer database was queried for patients with primary RP GCT who relapsed after first-line therapy between 1990-2024. Kaplan-Meier method was used to analyze progression free survival (PFS) and overall survival (OS). Survival outcomes based on type of second-line chemotherapy was compared using the log rank test.

Results

53 pts were included in the analysis. Median age at diagnosis was 33.7yrs (17.4-67.9). Primary tumor pathology was non-seminoma in 75.5% and seminoma in 24.5%. Predominant histology was seminoma (30.2%), mixed (26.4%), choriocarcinoma (17.0%), yolk sac tumor (13.2%), embryonal (9.4%), teratoma (3.8%). Other metastasis sites included pulmonary (56.6%), liver (35.9%), supradiaphragmatic lymph nodes (24.5%), posterior mediastinum (15.1%), pelvic lymph nodes (13.2%), brain (13.2%), bone (9.4%). IGCCCG risk was good in 30.2%, intermediate in 15.1%, and poor in 54.7%. First-line chemo was BEPx4 (54.6%), VIPx4 (11.3%), BEP x3 (7.5%), EPx4 (7.6%), BEPx2 + HDCTx2 (2.0%), and other (17.0%).

All 53 pts had progression of disease after first-line chemo. 43 received salvage chemotherapy, 5 received RPLND, 2 other salvage surgery, 1 radiation, and 2 received no salvage therapy. Salvage chemo was HDCT for 69.8% vs. standard salvage chemo for 30.2%. For pts treated with HDCT, 83% completed 2 cycles. 46.7% of those treated with HDCT progressed afterward. Of pts who had salvage RPLND, 3 were found to have teratoma and 2 had active GCT. 2 pts had other salvage surgery; 1 had thoracotomy with GCT and 1 had craniotomy with GCT. At time of last follow-up, 28.3% of all pts were alive with NED, 11.3% were alive with disease, 13.2% were lost to follow-up, and 47.2% had died of disease.

Table 1 lists PFS and OS by salvage chemo type.

Conclusions

Patients with relapsed primary RP GCT seem to have worse outcomes compared with historical results from relapsed gonadal GCT. A subset of patients with relapsed primary RP GCT are curable with salvage therapy.

	HDCT N=30	Standard dose chemo N= 13	 p-value
2-yr PFS	47.7% (95% 28.7-64.5)	31.8% (95% 7.7-59.9)	p=0.18
2yr OS	53.8% (95% 34.0-70.0)	68.6 (95% 30.5-88.7)	p=0.84

Translational/Clinical Research ***Resident Physician***

POSTER #166

EXERCISE HABIT FOR BREAST CANCER RISK REDUCTION (EXTENSION): FEASIBILITY AND EARLY EFFICACY OF A SINGLE-ARM PROSPECTIVE TRIAL

Niraj Shah[1], Donya Nemati[2], Danielle Halsey[1], Lina Sego[1], Tarah Ballinger[1]

[1] *Indiana University Melvin and Bren Simon Comprehensive Cancer Center*
[2] *Ohio State University*

Email: *shahnir@iu.edu*

Background: Women with a ≥20% lifetime risk of breast cancer are considered high-risk and benefit most from prevention strategies. While biomedical approaches like tamoxifen are common, behavioral interventions such as exercise remain underutilized, and many high-risk women do not meet physical activity (PA) guidelines despite evidence that PA reduces breast cancer risk by 10-25%. The EXTENSION program (EXercise habiT for brEast caNcer riSk reductION) was developed to promote exercise habits in high-risk women using the Individual and Family Self-Management Theory. This study assessed the feasibility and early efficacy of EXTENSION and explored biological correlates in serum and breast tissue.

Methods: This single-arm, prospective study enrolled women ages 18-69 with increased breast cancer risk who did not meet PA guidelines. Participants completed a 16-week online intervention delivered through the Canvas platform. The program included three 30-minute high-intensity interval training (HIIT) videos weekly and educational materials targeting self-regulation, self-efficacy, and habit formation. Feasibility was assessed via engagement with the online platform and study visit completion. Pre- and post-intervention assessments included: 1) behavioral questionnaires measuring self-regulation, habit formation, environmental cues, and social support; 2) subjective and objective PA using the Godin Leisure Time Exercise Questionnaire (GLTEQ) and wearable activity monitors; 3) body weight and composition via InBody 970 bioanalyzer; 4) serologic markers of inflammation; and 5) breast tissue biopsies collected through the IU Komen Tissue Bank. Pre- and post-intervention changes were compared using paired t-tests and Cohen's D to measure effect size.

Results: 35 participants were enrolled and 31 completed all pre- and post-intervention data collection. Completion rate for health behavior educational materials was 91.3% and 78.1% for the exercise videos. All theorized behavioral determinants increased post-intervention including intention (+0.14 (SE 0.04, d = 0.28)), habit (+0.55, SE 0.07, d = 0.56)), and self-efficacy (+0.55 (SE 0.03), d = 1.17). Paired t-tests revealed a significant reduction in total weight from pre- (M=187.41 lbs, SD = 36.3) to post-intervention (M=183.59 lbs, SD=37.55) (p = 0.03, Cohen's d = 0.35). Similarly, there was a significant reduction in percent body fat from pre- (M=41.71%, SD = 7.8) to post-intervention (M=40.55%, SD = 7.95) (p = 0.0013, Cohen's d = 0.59). The proportion of moderately and highly active individuals determined by GLTEQ scores increased from 41.9% (n=13) to 90.3% (n=28). Analysis of serologic and tissue changes is ongoing and will be reported separately.

Conclusions: The EXTENSION program is a scalable, online- delivered exercise program that demonstrates early feasibility and efficacy in motivating women at increased risk of breast cancer to increase PA. This led to significant reductions in weight and body fat that are associated with reduced risk. This work will be used to design larger implementation studies for at-risk women.

Translational/Clinical Research ***Senior Clinical Research Coordinator***

POSTER #167

ARTIFICIAL INTELLIGENCE PREDICTS 2021 WHO GLIOMA SUBTYPES FROM WHOLE SLIDE IMAGES

Shubham Innani[1], W. Robert Bell[2], MacLean Nasrallah[3], Bhakti Baheti[1,4], Spyridon Bakas[1]

[1] Division of Computational Pathology, Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis

[2] Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis

[3] Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

[4] Department of Biomedical Engineering, Emory University, Atlanta, GA, USA

Email: *sinnani@iu.edu*

Introduction : Diagnosis of adult diffuse glioma according to the WHO 2021 classification criteria mandate the integration of histologic features with molecular profiling. However, molecular profiling is expensive, time-demanding, and when not available leads to the 'not-otherwise-specified' status. We seek interpretable AI-based classification of glioma, as oligodendroglioma, astrocytoma, or glioblastoma, from H&E-stained slides .

Material and Method : We identified 2,114 multi-institutional whole slide images (WSIs), from two independent retrospective glioma collections, following their reclassification according to the WHO 2021 criteria: a) TCGA-GBM/TCGA-LGG (n_{WSI}=1,320, $n_{patients}$=654) & b) EBRAINS (n_{WSI}=$n_{patients}$=794). TCGA data are used for methodology development, whereas EBRAINS for independent hold-out validation. Each WSI undergoes comprehensive curation to account for any artifacts, such as tissue foldings, glass reflections, and pen markings. We then conduct a quantitative performance evaluation across: i) eight pathology-specific AI foundation models (FM) and an ImageNet-trained AI model that facilitate robust feature extraction, and ii) nine multiple-instance learning (MIL) approaches that are used to aggregate features into slide-level representations, to differentiate across the three glioma classes. Finally, we take into account magnification level combinations of the WSIs (2.5x, 5x, 10x, 20x), in an attempt to mimic the approach that expert neuropathologists follow to histologically assess tissue slides.

Results : Our approach yields AUC_{TCGA}=0.979 over a 10-fold cross-validation schema, and generalizable performance on the independent validation ($AUC_{EBRAINS}$=0.963), for the best performing FM and MIL in the multi-magnification setting. Our key findings indicate: i) domain-specific FMs outperform the ImageNet model, ii) MILs yield larger performance contributions when used with ImageNet models than with FMs, iii) Fusion of multiple magnifications adds value on both development and validation datasets. Interpretability analysis through attention heatmaps highlights distinct identifiable morphology features for each glioma class. Oligodendroglioma show uniform, round nuclei with perinuclear halos, microcystic and gemistocytic cells. Astrocytomas contain cells resembling astrocytes with irregular, elongated, or bipolar-shaped nuclei and fibrillary cytoplasmic processes. Glioblastomas display highly heterogeneous cellular composition, pleomorphic astrocyte-like cells, microvascular proliferation, multinucleated giant cells, and pseudo palisading necrotic regions.

Conclusion : Determination of glioma classes directly from routine clinically acquired H&E slides can obviate the need for molecular profiling, expedite conclusive diagnosis and hence clinical decision-making, even in underserved regions. Interpretability analysis towards distilled human-identifiable features can contribute in furthering our disease understanding.

Translational/Clinical Research ***Staff***

POSTER #168

CELL LIBRARY PROTOCOL AT INDIANA UNIVERSITY SIMON COMPREHENSIVE CANCER CENTER: RELOCATION OF FROZEN HUMAN SOLID TUMOR CELLS ACQUIRED FOR CLINICAL PROTOCOLS UTILIZING TUMOR VACCINES AND ADOPTIVE TRANSFER OF IN VITRO SENSITIZED T CELLS IN PATIENTS WITH METASTATIC CANCER.

Olivia DeHaven[1,2], Kayli Nguyen[1,2]

[1] *IUSOM*
[2] *IU Specimen Storage Facility*

Email: *odehaven@iu.edu*

Based upon murine studies using methylcholanthrene-induced sarcomas and tumor draining lymph node lymphocytes, Shu et Al. demonstrated T cell specific, anti -sarcoma activity in both weakly and non-immunogenic sarcomas. This led to the opening of the human cancer clinical trials 86-31, 92-81 and 93-76 at the University of Pittsburgh accruing 56 patients from 1986-2000.

Eligibility required ≥ 3 X10E8 sterile autologous viable tumor cells stored in liquid N2 for tumor cell vaccines and vaccine-draining lymph node lymphocyte (VDLNL)-tumor culture (IVS) and adoptive transfer with IL-2. Tumor was processed for eligibility from ~665 patients with melanoma, renal, adenocarcinoma (mostly GI), sarcoma and others. The samples were processed and stored at the IMCPL, a core facility and included tumor, lymphocytes (isolated from tumor, peripheral blood,VDLNL) and serum/plasma. The cells were relocated to Indiana University in 2008 and 2020 where the Cell Library protocol was IRB approved. ~ 7500 individual samples were relocated, organized and

Checked for identifiers and double checked against existing files. The data were captured in the Bio Specimen Storage Facility. The IU Cancer Center Tissue Procurement Group intends to confirm the transferred cell viability and sterility. This data set is unique given the maturity of outcomes, multiple cell types included, and large patient numbers. The availability at the IU Cell Library of viable, sterile cells that are autologous and allow for in vitro or in vivo studies of T cell-tumor interactions is a valuable resource. Its use may yield preclinical information that could be further explored in developing novel cell-based cancer therapies.

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

POSTER #169

ARTIFICIAL INTELLIGENCE-POWERED DETECTION OF SKIN CANCER USING BAYESIAN-OPTIMIZED ENSEMBLE CNNS

Michael Li[2,3], Jianneng Li[1]

[1] *Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, USA, Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN, USA, Notre Dame, IN*
[2] *Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, USA*
[3] *Department of GI Radiation Oncology, the University of Texas MD Anderson Cancer Center, Houston, TX, USA*

Email: *mli29@nd.edu*

Early detection of malignant skin lesions is critical for successful treatment outcomes. This novel deep learning framework presents an approach for automated binary classification of dermoscopic images using an ensemble of convolutional neural networks (CNN) with Bayesian hyperparameter tuning. A nested cross-validation framework (5 outer folds, 4 inner folds) is utilized to robustly evaluate model performance while preventing data leakage. The ensemble architecture integrated three pre-trained CNN models (ResNet101, EfficientNet-B4, DenseNet121) with custom attention mechanisms and learnable dynamic weighting of model outputs. Bayesian optimization identified optimal hyperparameters for data augmentation strategies, model architectures, and training procedures. The model was developed and evaluated using HAM10000 dataset (created by the Department of Dermatology at the Medical University of Vienna, Austria, and the skin cancer practice of Cliff Rosendahl in Queensland, Australia). To assess real-world generalizability, the final model was further validated on a completely independent external test set from Memorial Sloan Kettering Cancer Center (MSK). The nested cross-validation demonstrated dependable performance in select metrics, with an average AUC of 0.938 (±0.007), and accuracy of 0.911 (±0.004) across all folds. Furthermore, the model demonstrated strong generalization capabilities when tested on a completely external validation dataset from MSK. Each fold's optimized model maintained robust performance on this blinded test set, with only slight decreases in metrics: average accuracy of 0.873 (±0.005), AUC of 0.892 (±0.007), and specificity of 0.925 (±0.015). When combining all folds into a final ensemble, performance further improved to an AUC of 0.924, accuracy of 0.884, and specificity of 0.938. These results suggest that deep learning approaches hold potential as decision-support tools for dermatologists in skin lesion classification. Additional clinical validation studies are required.

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

POSTER #171

ANALYSIS OF MICROSEMINOPROTEIN-BETA BY IMMUNOHISTOCHEMISTRY IN CLINICAL TRIALS WITH QUPATH TO PROGNOSE PROSTATE CANCER

Andrew Myung[1]

[1] *Indiana University School of Medicine*

Email: *amyung@iu.edu*

High-quality evidence from randomized-control trials has demonstrated that patient outcomes do not differ significantly between treatment and active surveillance in low and moderate-risk disease. Accurate prognostic markers are needed to identify high-risk patients. Microseminoprotein-beta (MSMB) has been studied for its potential role in predicting prostate cancer progression, but previous histologic studies have been limited by qualitative staining assessments and tissue microarray-based approaches that do not capture expression heterogeneity.
Forty-seven Tissue sections from fourteen prostate cancer patients were selected with a variety of Gleason grades. Immunohistochemistry (IHC) for MSMB was analyzed. MSMB expression showed marked spatial heterogeneity across tumor and normal tissue regions, reinforcing the need for whole-organ assessments.
Follow-up data was extracted for the ISUP grade 5 patients, four of which had distant metastasis within five years, and four who did not. Future work will focus on expanding our dataset and building prognostic models associating heterogeneity with future metastatic disease and understanding the mechanisms by which normal tissue may play a role in tumor progression.

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

POSTER #172

TARGETING PRDX1 IN COMBINATION WITH REF-1 INHIBITION TO ENHANCE PDAC TREATMENT RESPONSE

Stuti Kaushalkumar Patel

Email: *stupatel@iu.edu*

Pancreatic Ductal Adenocarcinomas (PDACs) are among the most lethal malignancies, characterized by their aggressive nature and profound resistance to conventional therapies. A critical factor in this resistance is the metabolic and redox plasticity of PDAC cells, which allows them to adapt and survive under therapeutic stress. In this context, redox signaling regulators have emerged as promising therapeutic targets. This study focuses on the role of Peroxiredoxin 1 (PRDX1), a pivotal antioxidant enzyme involved in detoxifying hydrogen peroxide and maintaining redox balance, in modulating treatment response when combined with inhibition of the redox effector factor-1 (Ref-1).

A metabolic CRISPR-Cas9 screen conducted in MIAPaCa-2 cells revealed several metabolic vulnerabilities that synergize with the Ref-1 redox inhibitor APX2014. Among the top hits—PRDX1, G6PD, SEPHS2, TXNRD1, NADK, ALAD, and ALAS1—PRDX1 emerged as a particularly compelling candidate. PRDX1 plays a central role in protecting PDAC cells from oxidative stress by scavenging reactive oxygen species (ROS), thereby supporting survival under oxidative and therapeutic insults. Notably, knockdown of PRDX1, in combination with APX2014 treatment, resulted in a marked increase in cell death, suggesting a synergistic mechanism that disrupts the redox homeostasis essential for PDAC cell survival.

Current efforts are directed toward optimizing PRDX1 knockdown strategies using shRNA and CRISPR interference systems to precisely modulate its expression. Preliminary results indicate that PRDX1 depletion significantly elevates intracellular ROS levels, rendering PDAC cells more susceptible to Ref-1 inhibition and amplifying oxidative damage beyond repairable thresholds. Additionally, the impact of PRDX1 knockdown on downstream antioxidant pathways and metabolic networks is being characterized to delineate compensatory responses and identify further therapeutic targets.

Another target of interest, SEPHS2, has shown an additive, though not synergistic, effect with APX2014. However, PRDX1 remains the most promising due to its central redox regulatory function and direct involvement in modulating oxidative stress thresholds. Future directions include in vivo validation of the PRDX1 and Ref-1 co-targeting strategy, assessment of tumor regression, and evaluation of immune microenvironment alterations.

By elucidating the critical function of PRDX1 in PDAC survival and its interplay with Ref-1 redox signaling, this study aims to establish a foundation for novel combination therapies. These therapies would exploit redox vulnerabilities to overcome therapeutic resistance and improve treatment outcomes in PDAC patients.

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

POSTER #173

EPIGENETIC REPROGRAMMING OF STING PATHWAY: A PROMISING COMBINATION STRATEGY OF DNMT INHIBITORS AND STING AGONISTS IN HIGH_GRADE SEROUS OVARIAN CANCER

Saranya Rajendran[1], Elnaz Abassi Farid[1], Ishani Chattopadhayay[1], Shu Zhang[1], Kaushlendra Tripathi[2], Feyruz V. Rassool[2,3], Kenneth P. Nephew[1,4,5]

[1]Medical Sciences Program, Indiana University School of Medicine-Bloomington, Bloomington, IN 47405, USA

[2]University of Maryland Marlene and Stewart Greenebaum Comprehensive Cancer Center, Baltimore, MD 21201, USA.

[3]Division of Translational Radiation Sciences, Department of Radiation Oncology, University of Maryland School of Medicine, Baltimore, MD 21201, USA,

[4]Department of Anatomy, Cell Biology and Physiology, Indiana University School of Medicine Indianapolis, IN 46202, USA, 5Indiana University Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN 46202, USA

High-grade serous ovarian carcinoma (HGSOC), accounting for 70% of ovarian carcinoma cases, is often diagnosed at an advanced stage and associated with a poor prognosis. A significant clinical challenge is the lack of effective treatments for platinum-resistant OC and non-BRCA-mutated, homologous recombination repair proficient tumors, which represent over 50% of HGSOC. Furthermore, although cancer immunotherapies can produce complete and durable responses, response rates in HGSOC are modest with most patients experiencing only transient therapeutic benefit, underlining the urgent need for novel approaches to enhance antitumor immunity. Our prior research demonstrated that DNMT inhibitors (DNMTis) and PARP inhibitors (PARPis) upregulate ZNFX1, a zinc finger protein that facilitates STING-dependent interferon signaling and antitumor immune responses through a mechanism of viral mimicry. We hypothesize that in addition to DNMTis role in upregulating ZNFX1 and eventual antitumor immune responses, DNMTis restores STING through epigenetic reprogramming, leading to tumor cell intrinsic STING activation mediating anti-tumor response. In this study, we investigated the role of STING in HGSOC and the mechanisms underlying STING pathway activation by DNMTi. Analysis of the TCGA and GTEX database revealed reduced basal STING expression in HGSOC patient tumors, particularly in TP53 mutant HGSOC. Additionally, higher STING expression was linked to improved overall and progression free survival. Further analysis of STING methylation in normal and HGSOC samples from TCGA database identified 6 hypermethylated sites near the promoter region (or TSS). Similarly, Infinium Methylation EPIC Array v2.0 BeadChip analysis in the OVCAR3, a HGSOC cell line confirmed hypermethylation of these six sites. To determine whether this promoter-driven silencing could be reversed, we treated HR-deficient (HRD) and proficient (HRP) HGSOC cell lines with increasing concentrations of DNMTi. This treatment significantly increased STING expression in a dose-dependent manner, along with upregulation of downstream STING targets. To further evaluate if treatment with STING agonist could restore functional activation of STING signaling in addition to demethylation, we treated cells with DNMTi, STING agonist and combination and evaluated for downstream STING targets by western and qPCR. Notably, the combination of DNMTi and STING agonist significantly increased pSTING1, pTBK1, and pIRF3 and apoptosis-dependent cell death. Additionally, combination treatment markedly reduced oncogenic phenotypes compared to single drugs. Together, these results strengthen the idea of epigenetic regulation of STING expression in HGSOC, regardless of HRD status. Combining a DNMT inhibitor with a STING agonist has the potential to enhance the antitumor immune response and represents a promising therapeutic strategy for HGSOC patients.

Translational/Clinical Research ***Faculty***

POSTER #174

FLOW CYTOMETRY CORE

Maegan Capitano[1], Jim Ropa[1]

[1] *IU Simon Comprehensive Cancer Center, Indianapolis, IN*

Email: *malcapit@iu.edu*
jropa@iu.edu

POSTER #175

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Jill Henry[1], Anna Maria Stornolio[1]

[1] *IU Simon Comprehensive Cancer Center, Indianapolis, IN*

Email: *jihenry@iu.edu*
astornio@iu.edu

POSTER #176

IN VIVO THERAPEUTICS CORE

Tony Sinn[1], Karen Pollok[1]

[1] *IU Simon Comprehensive Cancer Center, Indianapolis, IN*

Email: *alsinn@iu.edu*
kpollok@iu.edu

POSTER #177

CELLULAR RESPONSE TECHNOLOGIES CORE

Emily Sims[1]

[1] *IU Simon Comprehensive Cancer Center, Indianapolis, IN*

Email: *ecwillar@iu.edu*

POSTER #178

TRANSLATIONAL RESEARCH CORE

Emily Sims[1]

[1] *IU Simon Comprehensive Cancer Center, Indianapolis, IN*

Email: *ecwillar@iu.edu*

POSTER #179

CENTER FOR PROTEOME ANALYSIS CORE

Mandy Bittner[1]

[1] *IU Simon Comprehensive Cancer Center, Indianapolis, IN*

Email: *mabitt@iu.edu*

POSTER #180

BIOINFORMATICS CORE

Jun Wan[1]

[1] *IU Simon Comprehensive Cancer Center, Indianapolis, IN*

Email: *junwan@iu.edu*

POSTER #181

BIOSTATISTICS AND DATA MANAGEMENT CORE

Patrick Monahan[1], Yong Zang[1]

[1] *IU Simon Comprehensive Cancer Center, Indianapolis, IN*

Email: *pmonahan@iu.edu*