MELVIN AND BREN SIMON CANCER CENTER
INDIANA UNIVERSITY

Cancer Research Day
2019 Abstract Book

POSTER #1

STRUCTURE AND FUNCTION OF LDB1-NUCLEATED TRANSCRIPTION FACTOR COMPLEXES IN T-CELL LEUKEMIA

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LIM domain binding protein-1 (LDB1) and LIM domain Only-2 (LMO2) are master regulator transcription factorsof hematopoietic stem and red blood cell development and are key drivers of human T-cell acute lymphoblastic leukemia (T-ALL). LDB1 and LMO2 exert their functions as components of a stable macromolecular assembly also containing Single Stranded DNA Binding proteins (SSBPs), as well as class II basic helix loop helix transcription factors: TAL1/SCL or LYL1, E-box proteins: E47/TCF3 or HEB/TCF12, and GATA factors. We analyzed steady state abundance and kinetic stability of LDB1, LMO2, and several other partners in T-ALL cell lines, via HALO epitope tagging in conjunction with FACS-based real-time live-cell analysis. We discovered a hierarchy of protein stability, with half lives in descending order: LDB1>SSBP>LMO2>TAL1>LYL1. Our results show that partner-partner interactions directly influence subunit stability and availability. Specifically, the stabilities of all protein partners were markedly prolonged by their incorporation into the LDB1/LMO2-nucleated complex. We identified lysine residues in LMO2 critical both for its stability and LDB1 binding, suggesting a mechanism for LDB1-dependent protection of LMO2 from ubiquitin/proteasomal degradation in T-ALL. As revealed by systematic in vivo reconstitution studies, co-expression of LDB1 and multiple partners is necessary to enforce a novel transcriptional profile in Jurkat cells. Our successful reconstitution of a LDB1-nucleated holocomplex enables us to further probe the structural and functional aspects of the entire assembly. Taken together, our studies provide insights into LDB1/LMO2 macromolecular complex formation and stability, with implications for understanding its role in red blood cell formation and for therapeutically targeting this complex in the highly treatment-resistant T-cell leukemia.

Basic Science ***Faculty***

POSTER #2

CPG-A AND CPG-B OLIGONUCLEOTIDES DIFFERENTIALLY REGULATE MR1-MEDIATED MICROBIAL AG PRESENTATION

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Mucosal-associated invariant T (MAIT) cells are conserved innate T cells that express a semi-invariant T cell receptor (TCR; Va7.2 in human and Va19 in mice). MAIT cells are activated by microbial Ags presented by the major histocompatibility complex (MHC)-related molecule, MR1. Little is known how MR1 molecules are processed and loaded with microbial Ag in APCs. We have previously found that Toll-like Receptor 9 (TLR9) is important in regulating MR1-mediated bacterial Ag presentation to MAIT cells in B cells. We further demonstrated that, similar to human B cells, CpG-A (but not CpG-B) upregulated MR1 expression on mouse B cells as well. CpG-A treatment increased MR1 expression on WT mouse B cells but not MR1 KO B cells, further confirming that activation of B cells by CpG-A specifically enhances MR1 surface expression. Initial RNAseq analysis has indicated that B cells activated by CpG-A are distinctively different from those activated by CpG-B or the vehicle control. In conclusion, CpG-A and CpG-B differentially impact MR1 expression and microbial Ag presentation. We have successfully deleted the expression of TLR9 and its downstream mediators in a human B lymphoblastoid cell line (B-LCL) using CRISPR technology. Future work will focus on how genes altered by the activation of CpG-A (but not CpG-B) regulate MR1-mediated microbial Ag presentation.

Basic Science ***Faculty***

POSTER #3

TREATMENT WITH SOLUBLE ACTIVIN RECEPTOR TYPE IIB ALTERS METABOLIC RESPONSE IN CHEMOTHERAPY-INDUCED CACHEXIA

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Cancer cachexia is a profound metabolic disorder that leads to perturbed energy balance and altered mitochondrial functioning. It has been shown that some chemotherapeutic agents can further exacerbate cachectic muscle wasting. ACVR2B/Fc is an inhibitor of activin receptor 2B signaling and has been shown to counteract loss of muscle mass in chemotherapy-induced cachexia. In this study we conducted a comprehensive, systems level metabolomics analysis to examine the effects of ACVR2B/Fc treatment on the metabolic phenotype of a murine model of chemotherapy-induce cachexia. Nuclear magnetic resonance and mass spectrometry-based metabolomics analyses were conducted on serum, muscle and liver tissues. In this study, mice were treated with Folfiri, ACVR2B/Fc and the combination. Folfiri treated mice presented a reduction in circulating glucose (-53%, 1.85e-5) that was not observed in the ACVR2B/Fc treated mice or those treated with the combination. A decrease in liver glucose (-46%, 0.003) was also observed in the Folfiri treated mice that was brought back to controls level with co-treatment with ACVR2B/Fc. Muscle lactate levels were increased (+40%, 0.033) in the Folfiri treated group, but at control levels with ACVR2B/Fc and co-treatment. These results suggest an increased systemic demand for glucose with Folfiri treatment that is at least partially corrected by co-treatment with ACVR2B/Fc. A significant reduction in a large panel of circulating lipids was observed in the Folfiri treated group, including lysophosphocholines, glycerophosphocholines and sphingomyelins. A more modest lipid reduction was observed with ACVR2B/Fc treatment while co-treatment yielded an intermediate effect. This metabolomics analysis shows that co-administration of ACVR2B/Fc with Folfiri yields a number of distinct metabolic effects that in some cases appear to counteract the perturbations induced by chemotherapy.

Basic Science ***Faculty***

POSTER #4

ALTERED RNA POLYMERASE II INTERACTOME DUE TO PAF COMPLEX PERTURBATION

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Misregulation of RNA Polymerase II (RNAPII) transcription has been implicated in numerous disease states, including cancer. Cancer cells have been denoted as transcription-addicted and depend upon upregulation of gene expression to survive. In order to understand how altered RNA polymerase II (RNAPII) transcription dynamics can lead to disease, the mechanisms that regulate steady-state transcription must first be understood. Characterizing these transcription mechanisms is required for identifying future therapeutic targets and disease treatments.

To better understand the workings of eukaryotic transcription, two proteins critical to transcription elongation were studied: PAF Complex (PAFC) subunits Cdc73 and Paf1. The PAFC is well-conserved, but its function is poorly-characterized. Study of Cdc73 and Paf1 has the potential to not only further understanding of normal transcription dynamics, but to also improve understanding of transcription in cancer, with potential to identify therapeutic targets. For example, the interaction between CDC73 (parafibromin) and MLL is required for leukemic cell proliferation. Moreover, PAFC overexpression has been shown to contribute to tumor development in non-small cell lung cancer and pancreatic cancer. However, further investigation of these elongation factors is necessary before considering therapeutic development. Severe elongation stress caused by inhibition or loss of an elongation factor, may induce DNA damage and further threaten cell health. Understanding the cell-wide effects of transcriptional stress caused by perturbation of an elongation factor is critical to any future development of transcription-targeting therapies.

To characterize the transcriptional stress caused by loss of Cdc73 or Paf1, *Saccharomyces cerevisiae* protein of interest deletion strains (*cdc73¿* and *paf1¿*) will be utilized. We hypothesize that loss of Cdc73, or Paf1, will alter the protein-protein interactions (interactome) of elongating RNAPII and cause an increase in stalled RNAPII thus resulting in shortened, improperly processed mRNAs and altered RNAPII accumulation and occupancy across the genome. Analysis of the changes in the RNAPII interactome following elongation factor disruption will shed light on the unique functions these proteins have within the basal transcription machinery.

Preliminary data from affinity purification mass spectrometry (AP-MS) analysis of RNAPII complexes shows altered protein-protein interactions within the RNAPII interactome in the absence of either Cdc73 or Paf1. These altered protein-protein interactions suggest loss of an elongation factor disrupts RNAPII interactions with other transcription regulatory proteins. The data also suggests that while the transcription machinery attempts to compensate for this loss, RNAPII continues to stall and/or arrest at a higher frequency, suggested by increased interactions with termination and degradation factors. Further analysis of the functional impact these altered interactions have both on the transcriptome and RNAPII accumulation and occupancy will further illuminate the functions of these elongation factors during transcription globally.

Basic Science ***Graduate Student***

POSTER #5

A SEMI-SUPERVISED DECONVOLUTION METHOD FOR QUANTIFYING THE COMPOSITION AND ACTIVITY OF TUMOR-INFILTRATING CELL TYPES

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Tumor microenvironment (TME) plays a key role in tumorigenesis, disease progression and acquirement of drug resistance. An accurate assessment of TME cellular compositions may not only shed light on the how TME interacts with cancer cells, but also bring new insights for translational researchers in studying the non-responding mechanism of immuno-therapy. Traditional deconvolution methods infer the relative proportions of predefined cell types based on a tissue omics data through either regression- or enrichment- based approaches. However, there are several challenges that remain unsolved in the current formulations, including (1) identifying the Immune/Stromal (I/S) cell types that truly exists in a TME, (2) identifying the marker genes for each cell type that are specifically expressed by one or a few I/S cell types in a TME, (3) co-linearity among to-be-assessed I/S proportions due to their co-infiltration. We have developed a novel semi-supervised deconvolution method namely ICTD (**I**nference of **C**ell **T**ypes and **D**econvolution), addressing the three challenges via (i) developing a Bi-Cross Validation (BCV) based matrix rank test to assess the significance level of the existence of cell types and signature genes, (ii) utilizing a constrained Non-negative Matrix Factorization (NMF) to eliminate the effect of co-linearity. We validated ICTD on bulk tumor data sets simulated using single-cell RNA-seq data. Our analysis suggested that ICTD has a largely improved prediction accuracy of TME compositions comparing to existing methods, and particularly, it is capable of identifying novel or sub-cell types. We applied our method to TCGA and other gene expression data of breast and prostate cancer. Subsets of CD8+ T cells with varied cytotoxicity levels and subtypes of fibroblast cells were identified. Moreover, integrated with an analysis on an independent single-cell and cell line gene expression dataset, we identified genes specifically expressed by cancer cells that are associated with decreased T cell cytotoxicity level.

Basic Science ***Graduate Student***

POSTER #6

CHARACTERIZATION OF ALDH1A1 INHIBITORS FOR CANCER THERAPY

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Cancer stem cells are subpopulations of cells capable of asymmetric division and tumorigenesis; as such, they are a possible driving force behind cancer recurrence, drug resistance and metastasis. In many solid cancer types, including breast, ovarian, lung and colorectal, cancer stem cells are characterized by elevated activity or expression of Aldehyde Dehydrogenase 1 (ALDH1) enzymes. Accordingly, ALDH1 expression has been clinically correlated with poor prognosis and increased drug resistance. ALDH1 is a family of enzymes that catalyze the NAD-dependent oxidation of reactive aldehydes, including retinaldehyde. More specifically, ALDH1A1 is often thought to be the dominant ALDH1 isoenzyme in breast and ovarian cancers. Nevertheless, the specific isoenzymes, substrates, and pathways that contribute to the role of ALDH1 in cancer are currently unknown, and the potential of ALDH1 enzymes as cancer therapy targets remains unverified in the clinic. The aim of this study is to develop and characterize isoenzyme-selective ALDH1A1 inhibitors that can be used as chemical tools to define the role of ALDH1A1 in cancer and confirm it as a therapeutic target.

This study consisted of kinetic assays, X-ray crystallography and cell culture assays to develop and characterize ALDH1A1 inhibitors based on two lead compounds: CM38 and CM10. A structure-activity relationship was built for each chemical scaffold, leading to the modulation of scaffold selectivity and development of potent bioactive analogues. ALDH1A1 inhibitors based on both scaffolds were shown to inhibit proliferation and invasion of breast cancer cell lines. The tumorsphere cell culture model, which is commonly used to enrich the cancer stem cell population, was used to further characterize the cellular activity of these compounds. Unlike traditional chemotherapeutics, such as taxol, which poorly target the cancer stem cell phenotype, ALDH1A1 inhibitors did not show a loss of potency in tumorsphere growth assays compared to assays in monolayer. An X-ray crystallography structure was determined for each scaffold bound to ALDH1A1. Based on the structural model, single-residue mutants of ALDH1A1 were designed to be resistant to inhibition by CM38 and CM10 scaffolds, but still enzymatically active. Expression of resistant mutants in a breast cancer cell line partially rescued the anti-proliferative effect of ALDH1A1 inhibitors, demonstrating specificity of inhibitor action in cells. In terms of therapeutic potential, one of the compounds showed synergy with Cisplatin, suggesting the possibility of combinatorial therapy. This work has led to the development of chemical tools for the study of ALDH1A1 and helped validate ALDH1A1 as a viable target in cancer, demonstrating a possible novel approach for targeting cancer stem cells.

Basic Science ***Graduate Student***

POSTER #7

TARGETING ERG THROUGH THE INNATE IMMUNE RESPONSE IN PROSTATE CANCER

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Prostate cancer is the second leading cause of cancer-related deaths in American men. Approximately half of all prostate cancers harbor a fusion between the highly expressed, androgen driven TMPRSS2 gene and the unexpressed ERG gene. This fusion places ERG under the active promoter region of TMPRSS2, resulting in aberrant ERG expression. This increases migration and invasion and coupled with secondary mutations, drives tumorigenesis. For this reason, targeting ERG has become a goal in prostate cancer research.

We performed an shRNA screen for genes required for ERG-mediated cell migration in prostate cells. This screen revealed that knockdowns in innate immune response genes produced less migratory cells. Based on this result, we tested the function of an inhibitor of the innate immune response. We found that this inhibitor can decrease migration and colony formation, but only in ERG-positive cells. This indicates a high degree of specificity for ERG function.

To address the mechanism by which ERG interacts with the innate immune response, we investigated signaling pathway components upon drug treatment. Our lab has shown that ERG must be phosphorylated by ERK to activate transcription, and the literature reports that PI3K-AKT signaling is important in the development of ERG-positive tumors. While no changes were observed in pAKT or pERK, pMEK and pERG levels are reduced. This provides a potential mechanism in which the innate immune response upregulates pMEK, leading to the phosphorylation and activation of ERG. This is supported by functional assays in which cells expressing a phosphomimetic ERG show no change in colony formation or migration when treated with the inhibitor. Additionally, ERG target genes are transcriptionally downregulated upon drug treatment in wild-type ERG expressing cells, but not in cells expressing the phosphomimetic ERG mutant.

To further explore the mechanism of this small molecule drug, we are investigating the drug's effect on ERG's ability to bind DNA as well as interact with co-activators and co-repressors. The drug seems to have no effect on the interaction between ERG and its canonical co-activator, EWS, but it does reduce ERG binding to DNA as observed through ChIP-qPCR. Additionally, we have preliminary data that suggests ERG is excluded from the nucleus in the presence of our inhibitor. These data begin to suggest a model in which the drug treatment not only prevents ERG from being activated by phosphorylation, but it also produces a shift in ERG localization from the nucleus to the cytoplasm.

A treatment that specifically targets ERG would be a large step in the clinical treatment of prostate cancer. It could aid patients suffering from metastatic, castration resistant cancer who have few alternative treatment options. Additionally, it could limit the metastatic potential of early stage cancer to slow progression of more aggressive cases.

Basic Science ***Graduate Student***

POSTER #8

THE ROLE OF ONCOSTATIN M IN THE PANCREATIC CANCER MACROENVIRONMENT

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Pancreatic cancer is the fourth leading cause of cancer deaths with a five-year survival rate of only 9%; pancreatic ductal adenocarcinoma (PDAC) comprises 90% of pancreatic cancer cases [1, 2]. PDAC tumors are highly desmoplastic and composed of cancer cells, cancer associated fibroblasts, vascular endothelial cells, and immune cells [1-3]. PDAC tumor cells express cytokines and elicit cytokine production from host tissues resulting in a systemic inflammatory syndrome. PDAC-induced hematologic abnormalities such as anemia, thrombocytosis, and increased neutrophil to lymphocyte ratio are associated with mortality. Cachexia is another morbidity associated with cancer marked by reduced body weight and fat and muscle wasting [4, 5]. These systemic consequences of PDAC are common and deadly. Cachexia itself affects 85% of PDAC cancer patients and leads to 25-30% of all cancer deaths. Most PDAC research centers on the tumor and its microenvironment, so understanding how PDAC tumors elicit inflammation in the macroenvironment of the host is critical to improve PDAC therapy and increase patient survival. Interleukin-6 (IL-6) is the best-studied inflammatory cytokine in PDAC and is important in PDAC tumor development, progression, immune surveillance, and cachexia [5, 6][7]. Much less is known about the other IL-6 family of cytokines, including Oncostatin M (OSM) [1, 9]. OSM is a secreted cytokine that functions in inflammatory disorders, cell proliferation, differentiation, and hematopoiesis. OSM binds GP130 and dimerizes with either OSM Receptor (Type II) or Leukemia Inhibitory Factor Receptor (Type I) to form the receptor complex and activate JAK/STAT signaling [8][9, 10]. OSM is secreted primarily by blood and immune cells, including monocytes and lymphocytes [11][8, 12][9, 13]. OSM regulates tissue remodeling, wound repair, and cytokine production including IL-6 from endothelial cells [2][14][15]. However, the roles of endogenous OSM in PDAC development, progression, and cachexia are still poorly described. We hypothesize that OSM and OSMR play key roles in the pancreatic cancer macroenvironment, including the PDAC microenvironment but also in abnormalities of blood, body composition, and tissue homeostasis. To study this, we will determine relevant phenotypes of Osm and Osmr in mice without cancer by using germline deleted mouse models and by exogenously expressing Osm by Aav-Osm in wildtype and *Il6* knockout mice. To investigate the relationship of Osm and Osmr, we will use an orthotopic PDAC cancer model to determine the roles of host Osm and Osmr in PDAC tumor growth, mortality, and PDAC-cachexia. The results of this study will provide evidence of the roles of OSM and OSMR in the pancreatic cancer macroenvironment.

Basic Science ***Graduate Student***

POSTER #9

IDENTIFYING GENES AND MICRORNAS THAT WHEN LOST, CAN POTENTIATE KRAS;P53-DRIVEN LUNG TUMORIGENESIS

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One of the main drivers of non-small cell lung cancer (NSCLC) is oncogenic *KRAS*, yet, mutant KRAS on its own fails to transform cells in culture or induce an aggressive tumorigenic phenotype *in vivo*. Importantly, even inclusion of p53 knockdown in the background of mutant *KRAS* still does not induce a fully transformed state in human bronchial epithelial cells (HBECs). This suggests that additional genetic insults are involved in NSCLC progression and that identification of these potentiators may lead to novel therapeutic agents. Indeed, therapeutically targeting KRAS or restoration of p53 have been met with multiple challenges culminating in a lack of therapeutics for *KRAS;p53* mutant tumors. Although tiny in size (20~22nt), microRNAs (miRNAs) are capable of negatively regulating a large number of protein-coding genes and, therefore, it is not surprising that their dysregulation is a major contributing factor to various human diseases including cancer. Importantly, global miRNA loss is prominent in many cancers and has been verified to promote lung tumor progression. This implies that down-regulation of certain tumor suppressive miRNAs may be responsible for transforming normal cells into a cancerous state. Therefore, we hypothesize that loss of certain miRNAs and relevant protein-coding genes can potentiate lung tumorigenesis that is driven by *KRAS* and/or p53. To address this hypothesis, functional genomic screens were performed using CRISPR-Cas9 in two models predisposed to cellular transformation: (1) The non-tumorigenic human bronchial epithelial cells that carry $KRAS^{G12V}$ and shRNA-p53 (HBEC-KP) and (2) the $Kras^{LSL\text{-}G12D}$ transgenic mouse model. In both models, the transgenic insults are not enough to promote full cellular transformation making both systems powerful models for the proposed study. In the first model, HBEC-KP cells were transduced with Cas9 and a lenti-sgRNA library, and the resulting cells with diverse gene knockouts were assayed for enhanced two-dimensional cell proliferation, acquired anchorage independence and *in vivo* tumorigenicity. Several sgRNAs were identified and validated to support anchorage-independent growth and in concordance with this, were enriched in two-dimensional culture over four-months of growth. In the second model, to identify genes that potentiate *Kras*-driven tumorigenesis, a *Kras;Cas9* double transgenic was generated. Both $Kras^{G12D}$ and Cas9 are induced specifically in the lungs following Cre-induced recombination via intratracheal delivery of adenovirus expressing Cre recombinase. At the same time a lenti-sgRNA library targeting murine protein-coding and miRNA-coding genes is delivered to induce targeted mutations. SgRNAs that are responsible for tumor progression will be significantly overrepresented in the resulting tumors and identified through deep sequencing. In-depth mechanistic studies will follow to understand how the identified miRNAs and genes interact with *KRAS* (murine model) and p53 (HBECs) to drive transformation of non-cancerous lung cells, which will ultimately pave the path towards developing more effective methods of cancer therapy.

Basic Science ***Graduate Student***

POSTER #10

DETERMINING THE ROLE OF BREAST CANCER CELLS IN FIBRONECTIN ACCUMULATION AT THE TUMOR SITE

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Tumor metastasis accounts for an estimated 90% of cancer related mortalities. During tumor progression, the extracellular matrix (ECM) protein fibronectin has been shown to contribute to the stiffening and alignment of the ECM at the primary tumor, and to also accumulate within the premetastatic niche before tumor cell dissemination. Interestingly, we observe that cancer cells produce elevated levels of soluble fibronectin. However, we hypothesize that the soluble fibronectin produced by breast cancer cells is not deposited into a matrix by the breast cancer cells, but is instead laid down by the resident fibroblasts to form the matrix. To test this hypothesis, we performed immunoblot analysis on the whole cell lysate, conditioned media, and extracellular matrix of 15 breast cancer cell lines. The selected cells represent various stages in the metastatic cascade, dormancy, as well as cells with fixed epithelial- and mesenchymal-like phenotypes. Only our control, human lung fibroblasts (HLFs), produced detectable levels of a fibronectin matrix. No detectable fibronectin deposition was observed for any of the breast cancer cell lines used, which was further confirmed using immunofluorescence. Interestingly, occasional spots of fibronectin were detected with immunofluorescence, which are attributed to the heterogeneity within the cell lines. Additionally, the immunoblot revealed that the majority of cell lines tested (10/15) secreted soluble fibronectin. Further, when HLFs were treated with exogenous fibronectin, they were able to lay down the exogenous fibronectin to create a fibronectin network. In two fibronectin knock-down HLF models (HLF KDs), the cells could not independently produce fibronectin. However, the HLF KDs could lay down soluble fibronectin obtained from media conditioned by breast cancer cells into a fibronectin network. Taken together, with our ongoing work on autocrine and exosomal fibronectin, we have strong support that breast cancer cells are not capable of independently laying down fibronectin in the extracellular matrix.

Basic Science ***Graduate Student***

POSTER #11

SSMD: A SEMI-SUPERVISED APPROACH FOR A ROBUST IDENTIFICATION OF CELL TYPES AND DECONVOLUTION OF MOUSE TRANSCRIPTOMICS DATA

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Multiple deconvolution methods have been developed for investigating the heterogeneous immune and stromal (I/S) cell types in human cancer tissue by deciphering their relative abundances using transcriptomic data. However, there is a lack of a user-friendly software for mouse transcriptomic data deconvolution. Key challenges for developing such a tool include: (1) limited training data sets for available for deriving signature genes of I/S cell types in sporadic mouse data; (2) mouse models of diverse geno-/pheno-types may have varied expressions of I/S cell marker genes; (3) the transcriptomic data may be collected from mouse with certain levels of immuno-deficiency; and (4) the transcriptomic data may come from highly diverse experimental platforms.

To solve these challenges, we (i) developed a novel non-parametric analysis method to derive potential I/S cell signature genes from a large collection of mouse data sets; (ii) implemented a low rank sub matrix identification method with a non-negative matrix factorization (NMF) based deconvolution method; and (iii) enabled the flexibility that certain I/S cell types may be absent if their respective cell markers do not form a significant low rank structure. The new method is applied to mouse prostate cancer data sets to infer the level of anti-cancer immune cell populations. Genes expressed by cancer cells that are negatively associated with the anti-cancer immune cells are further inferred, and compared with the results derived in human data. A user-friendly R package of the deconvolution method is released through GitHub.

Reference Format

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Basic Science ***Graduate Student***

POSTER #12

ADENOMATOUS POLYPOSIS COLI LIKE PROTEIN (APCLP) FUNCTIONS AS A NOVEL NEGATIVE REGULATOR OF NF-KB SIGNALING IN COLON CANCER CELLS

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Colon cancer (CRC) is the second leading cause of cancer related deaths in the United States. CRC is marked by aberrantly activated signaling of the nuclear factor of kappaB (NF-kB), a family of transcription factors which regulate wide varieties of cellular processes. Despite recent advances in comprehension of players of NF-kB signaling, deeper understanding of its regulation is imperative for the development of novel cancer therapeutics. Using the powerful validation-based insertional mutagenesis (VBIM) technique, we recently discovered APCLP as a novel negative regulator of NF-kB. The objective of this study is to elucidate the role APCLP in regulating NF-kB signaling in CRC at the molecular and biological levels and to understand the mechanism by which this regulation occurs. To determine the biological effect of APCLP on NF-kB signaling, we used lentiviral vectors to either overexpress or knockdown (shRNA) APCLP in human CRC cell lines (HT-29, HCT116, DLD-1). We show that overexpression of APCLP decreased the NF-kB activity, reduced cellular proliferation, migratory ability, as well as anchorage-independent growth of cells while knockdown of APCLP had an inverse effect. Furthermore, in vivo experiments in a xenograft mouse model confirmed that APCLP overexpression impeded whereas shRNA knockdown promoted tumor growth. To study the mechanism by which APCLP regulated NF-kB signaling, we conducted co-Immunoprecipitation experiments and confirmed that APCLP and the major subunit of NF-kB, p65, may complex or bind directly to each other. Studies are ongoing regarding the mechanism of interaction between APCLP and p65. In summary, discovery of APCLP and understanding of its molecular mechanism and biological function are significant because the knowledge acquired from this study could lead to utilization of APCLP as a potential biomarker and therapeutic target in CRC as well as other cancers that are driven by hyperactivated NF-kB.

Basic Science ***Graduate Student***

POSTER #13

CATALYTICALLY INACTIVE LSD1/COREST COMPLEXES DRIVE EMT IN PIK3CA MUTANT COLORECTAL CANCERS

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Activation of the Epithelial-Mesenchymal Transition (EMT) program is an essential mechanism for initiating cancer progression and metastasis. Chromatin modifying enzymes and various signaling pathways activate the EMT program. Lysine Specific Demethylase 1 (LSD1) is a chromatin modifying enzyme that is overexpressed in Colorectal cancer (CRC) and enhances cell migration. Activating mutations in the PI3K/AKT signaling pathway are observed in >40% of patients with CRC contributing to increased invasion and metastasis. Little is known with regard to how oncogenic signaling pathways synergize with chromatin modifiers to activate the EMT program and initiate the invasion-metastasis cascade. We discover that LSD1 expression is significantly elevated in CRC patients with mutational activation of PI3K compared to CRC patients with WT PI3K. We further demonstrate that LSD1 enhances activation of the AKT kinase in CRC cells, contextualizing the significance of this clinical observation. Perturbing LSD1 blocks AKT-mediated stabilization of the EMT-promoting transcription factor Snail, and effectively blocks AKT-mediated EMT and migration. LSD1 enhances AKT activation through a non-catalytic mechanism, as a scaffolding protein for the transcription-repressing CoREST complex. Our work is significant, as biomarkers for patient response to LSD1 inhibitors have remained elusive in CRC. Our data supports the hypothesis that inhibitors targeting the LSD1/CoREST complex may be clinically effective in CRC patients harboring PI3K mutation.

Basic Science ***Graduate Student***

POSTER #14

DELINEATING THE PHYSIOLOGICAL ROLE OF ADAR3 IN GLIOBLASTOMA

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Glioblastomas are considered to be one of the deadliest forms of malignancies known to man. With a median survival of no more than 15 months, the existing standard of care is associated with very poor prognosis. Therefore, advancements in understanding the underlying molecular mechanisms that contribute to glioblastoma progression could help us design potential therapeutic targets of great benefit. In the 21st century, a well-established fact is that cancer initiation, invasion and metastasis is driven by somatic mutations in the genome. Another mechanism to alter the cellular transcriptome and proteome is RNA editing; however, not much is known about the role editing plays in oncogenesis. Occurring in over 60% of human transcripts, A-to-I RNA editing is a type of RNA modification that alters the chemical and structural properties of the RNA. Catalyzed by the ADAR (Adenosine Deaminases Acting on dsRNAs) family of enzymes, hydrolytic deamination of adenosine produces inosine and is recognized as guanosine by all cellular machineries. This single nucleotide alteration can potentially affect RNA stability, RNA-binding, miRNA biosynthesis and regulation, translation, splicing and several other cellular processes involving RNAs.

Despite being structurally similar to ADAR enzymes, ADAR3 lacks functional deaminase activity. Studies have shown that ADAR3, which is exclusively expressed in the brain, negatively regulates editing and its absence leads to learning and memory impairment in mice. Moreover, we have shown that when compared to adjacent normal tissues isolated from the same patient, brain tumor samples have elevated ADAR3 protein levels. Despite these observations being reported, the physiological role of ADAR3 till date remains elusive.

Owing to its exclusive expression in the brain, preliminary studies in our lab have identified several ADAR3 targets that are dysregulated in glioblastomas. However, these targets were identified in a non-physiological ADAR3 overexpressed system. Thus, we are currently trying to identify RNA and protein targets of ADAR3 in human patient-derived glioblastoma cell lines that have endogenous levels of ADAR3. Furthermore, we are trying to CRISPR-knockout ADAR3 in these cell lines to study the molecular pathways that ADAR3 regulates. Consequently, this will help shed some valuable insight into the molecular mechanisms that ADAR3 regulates within glioblastomas allowing us to exploit ADAR3 as a potential biomarker or target for drug development.

Basic Science ***Graduate Student***

POSTER #15

TARGETING ALDH1A1 AND REGULATORY NETWORKS THAT SUPPORT STEMNESS IN OVARIAN CANCER

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Epithelial ovarian cancer is the deadliest amongst gynecologic cancers. Cancer recurrence is one of the major causes of mortality in ovarian cancer and is responsible for nearly 70% of deaths. A new paradigm explaining tumor relapse involves the persistence of cancer stem cells after chemotherapy. Ovarian cancer stem cells (OCSC) are hypothesized to be largely (or entirely) responsible for the emergence of chemoresistant tumors, and we have shown that OCSC contributes to recurrent, drug resistant high-grade serous ovarian cancer (HGSOC). Several biomarkers have been used to identify and characterize OCSC. Of these, aldehyde dehydrogenase (ALDH) activity has been shown to be a robust functional marker which is measurable by the Aldefluor assay. Of the ALDH isoforms, ALDH1A1 is an intracellular enzyme that oxidizes toxic aldehydes to carboxylic acids and plays a role in controlling differentiation pathways. High levels of ALDH1A1 expression have been associated with poor outcome in ovarian cancer patients. Our group and others have reported that ALDH1A1 high OCSC have a greater ability to seed ovarian tumors *in vivo*. However, the mechanism by which ALDH1A1 maintains stemness is unknown. We hypothesize that ALDH1A1 upregulation in OCSC is associated with genetic and epigenetic changes that mediate the cellular signals needed for OCSC to survive. To study the biology of OCSC regulation by ALDH1A1, we use compound 974, an ALDH1A1 specific small molecule inhibitor. Our preliminary data in several HGSOC cell lines such as OVCAR3, OVCAR5, Kuramochi, and PEO1 show that compound 974 can reduce ALDH enzyme activity ($p<0.01$) as measured by Aldefluor assay. CSC has the ability to survive as spheroids under anchorage dependent conditions in specialized stem cell medium, and treatment with compound 974 for 48 hours significantly reduced ($p<0.01$) spheroid formation in three cell lines representative of HGSOC (OVCAR3, OVCAR5, and PEO1). Compound 974 treatment also reduced the clonogenic survival of OVCAR3 ($p<0.001$) and PEO1 ($p<0.05$). Taken together, these data warrant further investigation into the molecular mechanism involved in the loss of stemness associated with ALDH1A1 inhibition. To better understand the gene networks altered by compound 974, we are performing global transcriptomic sequencing (RNA-seq) on the cells sorted based on ALDH activity into ALDH+ and ALDH- by fluorescence-activated cell sorting (FACS), treated with compound 974. We believe the information gained will give insights into the pathways involved in the regulation of stemness. This knowledge will be used to design therapeutic strategies to treat chemoresistant tumors to herald a better outcome in patients with late stage disease.

Basic Science ***Graduate Student***

POSTER #16

THE ANDROGEN RECEPTOR FUNCTIONS IN 3' END PROCESSING TO UPREGULATE AN ONCOGENIC ISOFORM

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The EWSR1 gene plays an important role in many cancer types. In Ewing's sarcoma, recurrent rearrangements involving the EWSR1 gene account for 95% of cases. The most common rearrangement produces the protein EWS-FLI which is a highly studied oncogenic driver. In prostate cancer, the protein encoded by the EWSR1 gene, EWS, is an essential promoter of oncogenesis. Therefore, understanding how the EWSR1 gene is regulated is important to understand cancer progression and could guide future therapeutic design.

Alternative RNA processing events produce numerous isoforms from a single gene that can have unique function. There are several annotated isoforms of *EWSR1*, yet only the longest isoforms (referred to as "full length") have been studied. Another *EWSR1* isoform, termed the N-terminal isoform as it encodes the N-terminus of EWS, has been identified in 3' sequencing data but has unknown function. The N-terminal isoform is generated by alternative polyadentylation and ends with an alternative last exon unique to this isoform. Interestingly, I found that the exons corresponding to the N-terminal isoform are upregulated when androgen receptor (AR) levels are high in primary prostate tumors. I then discovered that when stimulated with androgens, AR directly binds near the alternative last exon in prostate cancer cells. In patient tumor samples, AR also binds to this region, however this binding is not observed in matched adjacent normal tissue, suggesting AR binding to the EWSR1 gene is a tumor specific event. Interestingly, patients with the greatest AR enrichment at the tumor specific EWSR1 site had a more aggressive disease measured by pathological staining compared to patients with weaker AR enrichment. To determine if AR binding caused changes expression of the *EWSR1* isoform, I used isoform specific qRTPCR and found that AR induction by androgens resulted in upregulation of the N-terminal isoform.

Since the function of the N-terminal *EWSR1* isoform has not been elucidated in the literature, I overexpressed either the N-terminal isoform or the full length isoform in an androgen insensitive cell line and measured oncogenic potential. Strikingly, the cells expressing the N-terminal isoform robustly induced cell migration and colony formation compared to the cells expressing the full length isoform or an empty vector. These data suggest that AR binding to the EWSR1 gene directly upregulates an isoform with oncogenic function in prostate cancer.

While *EWS-FLI* has been observed in Ewing's sarcoma patients since the 1980s, and the function has been well described, the genesis of *EWS-FLI1* remains elusive. AR binding has been shown to generate a prominent gene fusion in prostate cancer. Strikingly, the region I found AR to bind in *EWSR1* is the breakpoint that leads to the *EWS-FLI1* fusion. This study, therefore, may provide insight to the genesis of *EWS-FLI1*.

Basic Science ***Graduate Student***

POSTER #17

TEMPERATURE SENSITIVE MUTANT PROTEOME PROFILING (TEMPP): A NOVEL TOOL TO ANALYZE THE IMPACTS OF MISSENSE MUTANTS ON THE PROTEOME

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Thousands of missense mutations have been associated with disease, including many cancers and neurological disorders. ~60% of these mutations have been predicted to affect protein stability and/or protein-protein interactions (PPIs). While genomic and transcriptomic sequencing data are abundant and readily accessible in databases such as The Cancer Genome Atlas, changes in mRNA have shown to explain only ~40% of protein level changes, thus, a full understanding of disease relies not only on genomic data, but also on defining the proteome. Many disease-causing proteins do have existing structural information, but these data cannot provide a complete picture of how protein function or PPIs are affected. Current methods of studying effects on protein function or PPIs require much time and some level of expertise. High-throughput methodology to evaluate how mutations in a single protein could affect PPI networks would greatly reduce the time and effort needed to characterize global mutant proteins and aid in the prediction of phenotypic outcomes resulting from genomic mutations. We have developed a tool coupling cellular thermal shift assay (CETSA) with mass spectrometry (MS) to study the effects of missense mutations on protein thermal stability on a global proteome level, which we have named Temperature sensitive Mutant Proteome Profiling (TeMPP). Yeast was used as a proof-of-principle model system with a particular focus on the ubiquitin-proteasome system (UPS). As the UPS is a key regulator of many proteins involved in critical cellular events, we hypothesized that perturbations within the proteasome complex would have a large impact on the proteome and would make an interesting subject to begin our studies. Furthermore, the proteasome's role in protein turnover and homeostasis has made it an appealing therapeutic target, especially in the treatment of multiple myeloma, therefore, characterizing its PPIs could be largely impactful to the cancer field. In the mutant strains studied —one within the core proteasome and one within the regulatory particle— we observed few proteins changing in thermal stability, despite global quantitative proteomics experiments revealing ~50% of proteins display significant changes in abundance. Overall, our results have shown that TeMPP is a high-throughput method with the capacity to resolve PPI effects of missense mutations with high specificity. Use of this method has a large potential to help build a foundation for the characterization of how missense mutations can affect cellular protein homeostasis and thereby lead to disease phenotypes.

Basic Science ***Graduate Student***

POSTER #18

FANCA MODULATES PLK1-BRCA2-BUBR1 AXIS DURING MITOSIS

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Fanconi anemia (FA) is a heritable bone marrow failure syndrome characterized by congenital skeletal malformations and predisposition to childhood acute myeloid leukemia. FA is caused by biallelic germline mutations of any one of the 21 known genes that comprise the FA pathway, which includes BRCA2 (FANCD1). Monoallelic loss of BRCA2 is associated with increased risk of breast and ovarian cancers. The tumor suppressive role of FA proteins in DNA damage repair is well established. However, a growing body of evidence suggests mitotic dysregulation in FA-deficient cells may contribute to the genomic instability that is a hallmark of FA. The poorly understood molecular mechanisms by which FA proteins safeguard cell division may represent an untapped avenue of therapeutic targeting in FA-deficient malignancies. To identify kinases that are synthetic lethal upon loss of *FANCA*, the most commonly mutated gene in FA, we performed a kinome-wide shRNA screen in patient-derived *FANCA-/-* fibroblasts. The screen identified a number of mitotic-regulating kinases, including PLK1 and BUBR1. Interaction between PLK1 and BRCA2 allows posttranslational modifications of BUBR1 that enable its stability and function in the spindle assembly checkpoint (SAC). We have previously reported that FANCA is required for BRCA2-mediated acetylation of BUBR1, which stabilizes BUBR1 during SAC. Others have shown that interaction between PLK1 and BRCA2 promotes phosphorylation of BUBR1 by PLK1 at interkinetochore tension sensitive site T680, which is required for proper chromosomal alignment at the metaphase plate. Here we report validation of synthetic lethality between FANCA and PLK1 using the clinically-approved small molecule inhibitor Volasertib. Further, we demonstrate that phosphorylation of BUBR1 at T680 is impaired in *FANCA-/-* cells. These findings reveal a dependency of FANCA-deficient cells on PLK1-BUBR1 signaling and provide further insight into the FANCA-BRCA2-BUBR1 signaling axis, providing preclinical rationale for targeting PLK1 in FANCA-deficient malignancies.

Basic Science ***Graduate Student***

POSTER #19

UNDERSTANDING ROLE OF ALTERED DNA DAMAGE RESPONSE IN SURVIVAL OF OVARIAN CANCER STEM CELLS AFTER TREATMENT WITH PT- THERAPY

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Majority of ovarian cancer cases are detected at late stage and patients with Stage IV disease only have a 17% survival rate. Chemotherapy and tumor de-bulking are mostly effective in treating early stage ovarian cancer but are not effective for late stage cases. Late stage discovery of ovarian cancer is a detrimental factor in a patient's treatment because they have over 70% risk of cancer re-occurrence (for stage III or IV) due to chemo-resistant cells. When ovarian cancer cells are treated with Platinum (Pt)- based chemotherapeutic drugs like cisplatin or carboplatin, the drugs form adducts with DNA. These adducts activate the DNA damage response (DDR). During DDR, a normal cell can undergo DNA repair, cell cycle arrest or apoptosis. In ovarian cancer, a subpopulation of cells called ovarian cancer stem cells (OCSCs) are known to preferentially survive after treatment with Pt- based chemotherapeutic drugs. OCSCs are enriched in disease relapses and responsible for platinum chemo-resistance. We hypothesize that OCSCs undergo DDR and have distinct cell cycle profile from non-OCSCs, providing them with survival advantage when treated with chemotherapy, and hence leads to cancer reoccurrence. Our overall goal is to determine other potential proteins or pathways in DNA repair that can be targeted to sensitize OCSCs to Pt-based chemotherapy.

Breast Cancer 1 (BRCA1), a tumor suppressor gene, plays an important role in regulating genes in DDR and has an active role in DNA repair. *BRCA1* mutation is common in ovarian cancer. Although, less than 10% of high grade serous ovarian cancer, the most aggressive type of ovarian cancer, have *BRCA1* mutation. *BRCA1* mutation leads to locus- specific loss of heterozygosity (LOH) which increases *BRCA1* mutant tumor's sensitivity to chemotherapy due to defective homologous repair pathway. But, research has shown that *BRCA1* mutant tumors may not always have locus- specific LOH. These tumors have significantly lower percentage of survival compared to patients with locus- specific LOH. Our preliminary results using tumors from a xenograft mouse study showed decrease in *BRCA1* RNA levels in residual OCSCs that survive after treatment with carboplatin. Additionally, we observed decreased levels of *BRCA1* protein and RNA levels after acute treatment with cisplatin in vitro. Furthermore, we found significant increase in *BRCA1* promoter methylation after acute treatment with cisplatin. Thus, we hypothesize that decrease in *BRCA1* protein levels promote cell survival in OCSCs in response to treatment with cisplatin. Our current focus is to determine how BRCA1 is regulated in OCSCs after treatment with chemotherapy and how OCSC's unique cell cycle profile plays a role in chemoresistance. This research will help to understand how to sensitize OCSCs so that they do not survive chemotherapy.

Basic Science ***Graduate Student***

POSTER #20

BMI1 AND RING1A ARE INVOLVED IN H2A UBIQUITINATION AT SITES OF PLATINUM-INDUCED DAMAGE

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Ovarian cancer (OC) is a lethal gynecological malignancy with a 5-year survival rate of 46%. Surgical debulking followed by platinum and taxane based chemotherapy is the standard of care for OC patients. However, the major obstacle in the use of these agents for treatment of OC patients is development of chemoresistance. An epigenetic mechanism commonly associated with development of platinum resistance is promoter DNA methylation and associated gene silencing. Transcriptional silencing of tumor suppressor genes like MutL homolog 1 (MLH1) by this mechanism has been well established in development of platinum resistance. However, the mechanism of initiation of this aberrant promoter DNA methylation is not known. Platinum agents are DNA damaging agents which crosslink guanines and form intra-strand or inter-strand adducts. We hypothesize that platinum induced DNA damage or repair of the damage results in recruitment of proteins involved in transcriptional repression to sites of damage. Recruitment of repressive proteins results in transient repression of transcription in the vicinity of damage to promote repair. However, similar to our findings with enzyme induced double strand breaks, repair of damage can occasionally result in retention of repressive proteins at sites of damage causing persistent transcriptional repression and gene silencing. Such persistent repression of key loci potentially contributes to the development of platinum resistance. We demonstrate that treatment of platinum sensitive OC cells with the IC_{50} dose of cisplatin for 8 hours resulted in ubiquitination of H2A/H2AX by western blot analysis. We hypothesize that this ubiquitination occurs on K119 of H2A/H2AX. H2A/H2AX ubiquitination at K119 mediated by Polycomb repressive complex 1 (PRC1) has been associated with transcriptional repression and gene silencing during development and differentiation and also during DNA repair. We observe a reduction in platinum induced H2AX ubiquitination on knockdown of PRC1 complex member RING1A using western blot analysis. Using immunofluorescence, we observe that BMI1, another PRC1 complex member, localizes to sites of platinum induced DNA damage. Platinum induced lesions are repaired by different repair pathways including – Nucleotide excision repair pathway (NER), Fanconi Anemia repair pathway (FA) and Homologous recombination repair (HRR). We demonstrate that knockdown of proteins in global genome NER and HRR pathways results in a decrease in platinum induced ubiquitination. Through on-going studies we seek to further elucidate the mechanism of recruitment of BMI1, RING1A to sites of platinum induced DNA damage and their role in long-term gene silencing. Understanding this mechanism will enable us to design inhibitors to prevent the occurrence of platinum resistant tumors in OC patients.

Basic Science ***Graduate Student***

POSTER #21

EXPLORING THE POTENTIAL TO TARGET GLUTAMINE CATABOLIC FLUX IN MYC DRIVEN LYMPHOMAS TO INHIBIT MYC FUNCTION

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Oncogenic signals have been shown to significantly alter the metabolism profiles of tumors. This change in metabolic profile is also dictated by tissue of origin, type of driver mutations, vascular supply, dietary profile and therapy. Numerous studies, including ours have shown the dependency of key oncogenic signals on nutrient availability. These metabolic dependencies present unique targets for therapeutic intervention.

MYC dysregulation is seen in over 50% of cancers and is a key player in many lymphoid malignancies. In Burkitt's lymphomas, MYC expression is regulated by heavy chain enhancers through a t(8;14) (q24;q32) chromosomal translocation, leading to high ectopic expression of MYC. We have data to show that even during nutrient limitations, Myc transcript levels remain unchanged. This and recently published data on the ineffectiveness of transcription inhibitors point to the fact that targeting translation would provide better clinical outcomes.

Glutamine is a conditionally essential amino acids and maintained at high levels in the circulating blood. Glutamine catabolism is frequently upregulated in a variety of cancer cells, especially MYC driven cancers and is used to replenish TCA intermediates driving biosynthesis of various classes of macromolecules. Our preliminary data, including RNA sequencing data from CCLE suggests that MYC driven lymphomas are very sensitive to glutamine deprivation. We observed acute destabilization of MYC protein levels without any changes in MYC transcription. To check whether we can target the MYC protein levels by targeting the downstream catabolic flux, we used a pan transaminase inhibitor amino oxyacetate. Using the inhibitor, we found acute depletion of MYC protein levels, independent of its mRNA. We also observed a partial rescue of this phenotype by supplementing with aspartate, a key precursor in both protein and nucleotide biosynthesis. This points to the critical role of aspartate transaminase (AST) 1/2 in these lymphoma cells.

Our future directions include teasing apart the role of aspartate transaminases (cytosolic and mitochondrial) and the role of alpha ketoglutarate and identifying the mechanisms by which aspartate regulates MYC translation.

Basic Science ***Graduate Student***

POSTER #22

ACTIVATION OF THE ONCOGENE ERG BY THE RAS/ERK AND PI3K/AKT PATHWAYS

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The TMPRSS2-ERG re-arrangement occurs in ~50% of prostate cancers and results in expression of full length or N-terminally truncated (¿32) ERG protein in prostate epithelia. ERG is not normally expressed in the prostate and upon expression it promotes tumorigenesis via altered gene expression, stimulating epithelial-mesenchymal transition, cellular migration/invasion, and transformation. ERK-mediated phosphorylation of ERG is required for ERG function in prostate cells, but the reason for this requirement is unknown. Here, we report a mechanism whereby ERK-mediated phosphorylation of ERG at S96 results in dissociation of EZH2 and SUZ12 components of the polycomb repressive complex 2 (PRC2) and allows for ERG target gene activation. Conversely, loss of ERG phosphorylation at S96 resulted in recruitment of EZH2 across the ERG-cistrome and a genome-wide loss of ERG-mediate transcriptional activity.

ERG has previously been demonstrated to co-operate with the PI3K/AKT signaling pathway in the formation of tumors in the prostate and AKT activation is necessary for ERG mediated tumorigenesis in a mouse xenograft model. Although activation of the PI3K/AKT pathway is understood to be necessary for tumorigenesis the exact molecular mechanism of this requirement is not fully understood. We have previously found that AKT does not directly phosphorylate ERG in vitro and in prostate cells. In order to determine how the Ras/ERK and PI3K/AKT pathways interact with each other in ERG mediated phenotypes and tumorigenesis, we expressed ERG phospho-mutants in combination with activated AKT in prostate cells and subjected them to functional assays. Strikingly, we found that phospho-mimetic ERG S96 abrogated the requirement for AKT activation in tumorigenesis in a mouse xenograft model. To further interrogate this result, we conducted RNA-Seq in these cell lines to find common ERG activated genes in cells capable of tumorigenesis. Taken together, these results suggest a possible common molecular mechanism between the Ras/ERK and PI3K/AKT pathways in ERG mediated transformation which will be interrogated in further studies.

Basic Science ***Graduate Student***

POSTER #23

TUMOR-SPECIFIC CHEMOGENOMIC LIBRARIES BY STRUCTURE-BASED ENRICHMENT FOR GLIOBLASTOMA PHENOTYPIC SCREENING

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Solid incurable tumors like glioblastoma multiforme (GBM) are driven by multiple phenotypes such as uncontrolled growth, invasion, angiogenesis, and immune suppression. Suppressing most or all of these phenotypes with a small molecule will require compounds that selectively modulate multiple targets across several signaling pathways. Phenotypic screening holds substantial promise for the identification of such compounds, but the challenges associated with screening large collections of compounds in multiple assays using sophisticated spheroid and organoid assays limit the usefulness of the approach. Here, we propose a data-driven rational approach for the creation of tumor-specific chemogenomic libraries for phenotypic screening. These chemogenomic libraries are created by molecular docking of large chemical libraries to a collection of tumor-specific targets selected using tumor RNA-seq, protein-protein interaction, and protein structural data. Phenotypic screening of these enriched libraries led to several active compounds. Among them compound **1** (IPR-2025), which (i) inhibited cell viability of low-passage patient-derived GBM spheroids with single-digit micromolar IC_{50}s; (ii) blocked tube-formation of endothelial cells in Matrigel with sub-micromolar IC_{50}s; and (iii) had no effect on primary hematopoietic $CD34^+$ progenitor or astrocyte cell viability. RNA sequencing (RNA-seq) provided potential mechanism of action of **1** and mass spectrometry-based thermal proteome profiling (TPP) revealed multiple possible targets that included the scaffold protein RACK1, which was among targets predicted by molecular docking. Follow-up studies revealed that **1** engaged RACK1 in cancer cells, but no direct binding was observed with recombinant RACK1. The ability of **1** to inhibit GBM phenotypes without affecting normal cell viability suggests that our approach to create tumor-specific chemogenomic libraries may hold promise for developing more efficacious treatments for incurable diseases like GBM.

Basic Science ***Graduate Student***

POSTER #24

CHARACTERIZATION OF ENHANCER RNAS IN HUMAN ADAPTIVE IMMUNE CELLS

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Enhancer RNAs (eRNAs) are non-coding RNAs transcribed from active enhancer regions with an increasingly-recognized role in regulating gene transcription. Several studies have illustrated the role of eRNAs in tissue development and human diseases. However, their function in adaptive immune cells has been poorly explored. Here, we identify >2000 reproducible intergenic eRNAs in B cells, CD4+, and CD8+ T cells by integrating genome-wide DNA accessibility (ATAC-seq) and ribo-depleted stranded RNA sequencing data. These eRNAs were specific to immune cells and not present in other tissues. Over 60% of eRNAs were only expressed in one cell type, while other 20% were shared between CD4+ and CD8+ T cells, but absent in B cells. We show that eRNAs are distinct from non-transcribed enhancers and promoters by having the highest level of active and the lowest level of repressive histone modifications. eRNAs were highly enriched in super-enhancer architecture. As expected, eRNA expression correlated strongly with the transcription factor (TF) ChIP-seq signal of histone acyltransferases (e.g. GCN5), RNA polymerases (e.g. TAF7, GTF2F1), and chromatin regulators (e.g. MTA3, BRG1). We also identified other TFs (e.g. PML, NFATC1) that were highly correlated with eRNA expression, suggesting that they also potentially regulate eRNA expression. The expression of genes neighboring eRNAs was >10 fold higher than genes adjacent to non-transcribed enhancers, indicating that these genes are likely targeted by eRNAs themselves. In addition, these genes were involved in lymphocyte activation. Collectively, our work provides a comprehensive characterization of eRNAs in human adaptive immune cells.

Basic Science ***Graduate Student***

POSTER #25

TARGETING DAB2IP-MEDIATED WNT SIGNALING PATHWAYS IN OVARIAN CANCER STEM CELLS

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Ovarian cancer (OC) recurrence after tumor eradication by chemotherapy invariably heralds poor outcome. Recent data point to persistence of quiescent cancer stem cells (CSCs) not eliminated by chemotherapy and able to regenerate tumors as the main contributor to tumor relapse. Downregulation of tumor suppressor gene (TSG) DAB2IP, a member of the Ras GTPase-activating protein family, significantly correlated with poor patient survival in high-grade serous (HGS) OC. Furthermore, loss of DAB2IP in prostate and colon cancer enriched CSC population, suggesting a key role for DAB2IP in modulating cancer stemness. In the current study, we tested the hypothesis that targeting DAB2IP would inhibit CSCs in OC and prevent disease recurrence. Subpopulations of CSC and non-CSC were isolated from Kuramochi, OVCAR3 and COV362 HGSOC cell lines by fluorescence-activated cell sorting (FACS) based on aldehyde dehydrogenase (ALDH) activity, a consistent CSC marker. Expression of DAB2IP in ALDH(+) cells was lower ($P<0.05$) compared to non-CSC ALDH(-) cells. Chromatin immunoprecipitation (ChIP) analysis revealed greater ($P<0.05$) enrichment of H3K27me3 at DAB2IP promoter loci in CSC than non-CSC, and inhibiting EZH2 increased ($P<0.05$) DAB2IP expression in OC cells, suggesting that DAB2IP downregulation in CSC is caused by EZH2 methylation. Knocking out DAB2IP using CRISPR/Cas9 system in OC cell lines increased ($P<0.05$) ALDH1 expression and OCSC population. Enforced overexpression of DAB2IP decreased ($P<0.05$) the number of ALDH(+) cells, inhibited ($P<0.05$) the ability of these cells to form spheroids (14-day incubation under stem cell conditions) and decreased ($P<0.05$) colony formation. Furthermore, elevated DAB2IP expression decreased ($P<0.05$) cisplatin IC50 of both OCSCs and HGSOC cells and inhibited ($P<0.05$) cell migration capacity (Bowden chamber transwell assay), suggesting DAB2IP plays a role in regulating OCSC function. Mechanically, OVCAR3 cells and DAB2IP-overexpressing OVCAR3 cells were further analyzed by RNA-sequencing and bioinformatics. This transcriptome analysis revealed that DAB2IP overexpression resulted in significantly (FDR < 0.05, fold change > 2) altered expression of 449 genes, including markers strongly associated with CSC phenotypes, including down-regulation of *ALDH1A1, LGR5, PROM1, TWIST1* and ATP-binding cassette transporters. Ingenuity Pathway Analysis (IPA) for upstream regulators of differentially expressed genes revealed Wnt-signaling as a dominant pathway mediating the anti-OCSC effects of DAB2IP. Based on RNA-sequencing analysis, WNT5B expression decreased ($P<0.05$) by 3.83 fold, indicating that DAB2IP may negatively regulate Wnt signaling pathway by repressing WNT5B. In addition, treating OVCAR3 cells with Rac1 inhibitor CAS1090893, that inhibits non-canonical Wnt signaling, significantly decreased ($P<0.05$) the CSC population by 60%. Collectively, our data reveal that DAB2IP via Wnt-mediated signaling pathway plays a critical role in modulating CSC properties. Based on these novel findings, we are testing novel combination treatment strategies targeting OCSCs and with the goal of inhibiting tumor relapse and overcoming chemoresistance.

Basic Science ***Graduate Student***

POSTER #26

THE ROLE OF GLIOMA SECRETED CCL21 IN PLASMACYTOID DENDRITIC CELLS (PDC) RECRUITMENT AND TRANSCRIPTIONAL MODIFICATION OF MHC II IN PDCS.

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Background: Glioblastoma (GBM) is the most common type of primary tumor in the brain with poor prognosis. Despite its confinement to the brain by the blood brain barrier, it is a notorious evader and suppressor of the immune system. GBM creates an immunosuppressive tumor microenvironment (TME) by inducing a protumoral phenotype on tumor infiltrating immune cells such as macrophages, dendritic cells, and T cells. Previous studies have shown increased number of plasmacytoid dendritic cells (pDCs) in the TME displaying immunosuppressive characteristics with decreased secretion of IFN-a and increased expression of MHC-II molecule. The pDCs have been shown to be attracted by chemokine CCL21 secreted by GBM. Gaining a better understand of the mechanism behind the immunosuppressive characteristics of pDCs could be used to augment the current immunotherapies to improve survival outcomes in GBM patients.

Hypothesis: We hypothesize that GBM secretes CCL21 to recruits pDCs via ACKR3 and uses ACKR 4 signaling pathway to transcriptionally upregulates MHC-II.

Methods: Proteome profile assay and immunofluorescence were done *in vitro*, and immunohistochemistry of GL261 mice brain sections were perform to identify the presence of CCL21. *In vitro* migration of pDCs were done with varying concentrations of CCL21 to demonstrate CCL21 mediated migration of pDCs. 400,000 GL261 tumor cells suspended in PBS were injected into C57BL/6 mice intracranially. GL261. The lymphocytes from these mice were compared with that of the control group who did not go through any intracranial tumor cell injection. All of the mice were harvested one week post-operation (wpo) and the lymphocytes were isolated from brain, cervical lymph nodes, peripheral blood, and spleen. The Lymphocytes analyzed using flow cytometry after staining with CD45, CD11c, BST2, ACKR3, ACKR4 and MHC-II.

Results: CCL21 expression was identified in GBM. The migration study showed the chemotactic influence CCL21 has on pDCs. Accordingly, higher number of pDCs were found in the brains of GL261-injected mice compared to the control group. In addition, these pDCs expressed higher levels of ACKR4 and MHC-II compared to pDCs from control brain pDCs and tumor brain mDCs.

Conclusions: GBM recruits pDCs and manipulates them into displaying an immunosuppressive phenotype through the secretion of CCL21, creating a protumoral TME. Future studies include identifying the upregulation intracellular signaling markers as well as the use of functional CCL21 antibodies to prevent this phenomenon.

Basic Science ***Medical Student***

POSTER #27

NEUROPILIN 1 IS REQUIRED FOR FIBROBLAST GROWTH FACTOR RECEPTOR-MEDIATED TUMOR PROGRESSION

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Background: Human epidermal growth factor receptor 2 (ErbB2)-amplified Breast cancers initially response to treatment with targeted antibodies and kinase inhibitors (collectively called ErbB inhibition or ErbBi) at a high rate, but ultimately these tumors acquire resistance and undergo metastatic recurrence. Thus, there is a critical need to uncover metastatic events that drive the failure of ErbBi therapy. We have recently performed global gene expression analyses on several ErbBi resistant cell lines as compared to their drug sensitive counterparts. These data demonstrate induction of epithelial-mesenchymal transition (EMT) which included enhanced expression of fibroblast growth factor receptor 1 (FGFR1) and the axonal guidance molecules known as neuropilins (NRP). NRP1 is transmembrane receptor protein that functions as a co-receptor for a number of extracellular ligands including some isoforms of VEGF, class 3 semaphorins, HGF, FGF and TGF-ß. Several recent studies clearly link FGFR1 and Nrp1 to drug resistance and metastasis. Expression of Nrp1 is elevated in more invasive and advanced stages of cancer and the expression of Nrp1 in human breast cancer tissue negatively correlates with patient survival. We therefore sought to address the hypothesis that EMT-driven tumor metastasis is facilitated by an Nrp1:FGFR1 matrixsensing signaling complex. We also aim to determine if pharmacological disruption of this complex can overcome drug resistance and metastasis.

Methods and Results: Differential gene expression was compared from several datasets characterizing ErbBi sensitive and resistant cells, yielding enhanced expression of FGFR1 and Nrp1. Immunoprecipitation of FGFR1 coupled with mass spectroscopy indicated that FGFR1 forms a physical complex with Nrp and integrins upon induction of EMT. These data were confirmed by direct co-immunoprecipitation assays showing that FGFR1 and Nrp1 form a physical complex that also contains ß3-integrin. Formation of this ternary complex is dependent on the expression of Nrp1 as depleting or inhibiting Nrp1 disrupts these interactions and destabilizes basal levels of FGFR1. Our preliminary findings indicate that Nrp1 expression can be decreased via pharmacological inhibition of chromatin reader protein, bromodomain containing 4 (Brd4). Use of Nrp1 blocking antibodies or shRNA-mediated depletion of Nrp1 alone or in combination with an FGFR1 kinase inhibitor significantly inhibited Erk signaling and reduced tumor growth *in vitro* and *in vivo*. Our data indicate that depletion of Nrp1 not only inhibited tumor growth in the lungs but also enhanced the antitumor effects of FGFR kinase inhibitors.

RESULTS: Overall our studies indicate that Nrp1 facilitates aberrant FGFR signaling during EMT-associated drug resistance and metastasis. Pharmacological targeting of these key factors may provide improved outcomes for late stage breast cancer patients.

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

POSTER #28

CMET INHIBITION POTENTIATES THE TUMOR-SELECTIVE DAMAGING EFFECTS OF NQO1-BIOACTIVATABLE AGENTS BY COMPROMISING DNA REPAIR

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BACKGROUND/SIGNIFICANCE: In many solid tumors, overexpression of mesenchymal-epithelial transition factor (cMET) receptor tyrosine kinase and NADPH:Quinone oxidoreductase 1 (NQO1) correlate with poor prognosis. Studies have shown that high cMET and NQO1 expression in cancers promote tumor development, progression, metastasis, and acquired drug resistance through mechanisms that remain unclear. Recent studies have implicated cMET in the activation of PARP1, a critical factor involved in DNA damage response and repair. Therefore, targeting cMET is an attractive strategy for cancer therapy. However, the overall efficacies of cMET inhibitors are limited due to dose-limiting toxicities and lack of tumor selectivity as a monotherapy. Thus, we hypothesize that rational combination treatments with low-dose cMET inhibitors and NQO1-bioactivatable agents to selectively create DNA damage in NQO1+ cancer cells might ultimately provide maximal clinical benefit highlighting the novel role of cMET in DNA repair.

METHODS: Relative survival assays were assessed by long-term DNA assay to determine IC_{50} values in several cell line models. We investigated the molecular mechanism of action and synergy using Western blot and immunofluorescence techniques in several cell lines with pharmacological and genetic alterations in NQO1 and cMET expression/activity.

RESULTS: An NQO1-dependent synergistic lethality in cancer cells was induced by combination treatment with sublethal doses of ß-lapachone (ß-lap, an NQO1-bioactivatable drug in clinical trials) and cMET inhibitors (some in clinical trials and FDA-approved) when compared to vehicle control and single agent treatment. Mechanistically, a sublethal dose of ß-lap creates reactive oxygen species (ROS) that damage DNA nucleobases (e.g., 8-oxoguanine) but rapidly gets repaired by the ability of ROS-activated cMET to enhance PARP1 activity for efficient DNA damage repair in NQO1+ cells. Thus, when combined with cMET inhibition that compromises DNA repair, lethal double-strand breaks accumulate leading to the selective demise of NQO1+ cancer cells.

CONCLUSIONS: The mechanistic insights gained from our studies should allow re-purposing of FDA-approved cMET inhibitors in combination with NQO1-bioactivatable drugs to lower dose-limiting toxicities of both agents. Our results add a new strategy for personalized therapy: the targeting of cMET in NQO1+ cancers to potentiate the toxic effects of sub-lethal doses of NQO1-bioativatable agents and cMET inhibitors.

Keywords: ß-Lapachone, NQO1, ROS, cMET, PARP1, DNA repair

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

POSTER #29

SMALL-MOLECULE COVALENT MODIFICATION OF CONSERVED CYSTEINE LEADS TO ALLOSTERIC INHIBITION OF THE TEAD·YAP PROTEIN-PROTEIN INTERACTION

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The Hippo pathway coordinates extracellular signals onto the control of tissue homeostasis and organ size. Hippo signaling primarily regulates the ability of Yap1 to bind and co-activate TEA domain (TEAD) transcription factors. Yap1 tightly binds to TEAD4 via a large flat interface, making the development of small-molecule orthosteric inhibitors highly challenging. Here, we report small-molecule TEAD·Yap inhibitors that rapidly and selectively form a covalent bond with a conserved cysteine located within the unique deep hydrophobic palmitate-binding pocket of TEADs. Inhibition of TEAD4 binding to Yap1 by these compounds was irreversible and occurred on a longer time scale. In mammalian cells, the compounds formed a covalent complex with TEAD4, inhibited its binding to Yap1, blocked its transcriptional activity, and suppressed expression of connective tissue growth factor. The compounds inhibited cell viability of patient-derived glioblastoma spheroids, making them suitable as chemical probes to explore Hippo signaling in cancer.

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

POSTER #30

INTERACTION OF FANCA WITH SIK2 IN REGULATION ON MITOSIS

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Background

Fanconi anemia (FA) is a rare genetic disorders characterized with progressive bone marrow failure and congenital malformations. Genetically, it is caused by biallelic mutations on at least one of the FA genes, whereas clinically patients have a high predisposition to cancers such as acute myeloid leukemia. FA family members constitute an important pathway in repairing DNA interstrand crosslinks. Defects in this pathway render genomic instability and a higher chance of cancer. FA complementation group A (FANCA) mutations are present in 65% of FA patients. Besides being a subunit in FA core complex to tackle DNA damage, FANCA participates in regulating mitosis. However, the underlying mechanism is less defined. Further understanding on the functional roles of FANCA in mitosis not only benefit the FA patients but also those with diseases having FA pathway dysregulation.

Methods & Results

By using FANCA-/- patient fibroblast and its FANCA corrected counterpart (FANCA+/+), we identify Salt-inducible kinase (SIK) 2 as a novel partner that can interact with FANCA. SIK2 regulates centrosome separation during mitosis which is also crucial for the integrity of genome. Through immunofluorescence microscopy, FANCA and SIK2 were shown to co-localize in centrosome during mitosis. Co-immunoprecipitation also indicated the direct binding between FANCA and SIK2. Moreover, we detected the presence of SIK2 in kinetochore where FANCA was also reported to localize, strongly supporting that FANCA and SIK2 have functional connection and may involve in regulating mitosis. SIk2 possesses multiple phosphorylation sites among which the Serine 358 residue phosphorylation is highly correlated with the SIK2 activity, and such phosphorylation may associate with the mitotic progression. Knockdown of SIK2 with siRNA showed to slow down fibroblasts growth compared to cells treated with scramble control, corroborating the importance of SIK2 in cell proliferation. FANCA-/- patient fibroblasts showed lower pS358 SIK2 level than FANCA+/+ cells, suggesting that the lower proliferation rate observed in FANCA-/- fibroblast may be attributed to an less active SIK2 pathway. Proteasomal degradation, nevertheless, contributes to the low phospho-SIK2 level in FANCA-/- cells as the pS358 expression increased after MG132, a proteasome inhibitor, was added.

Summary & Perspective

Altogether, SIK2 is identified as a novel functional molecule for FANCA. SIK2 activity may be crucial for the proliferation of FANCA deficient cells. Further investigation on the effects of SIK2 inhibition using small molecule inhibitors may be useful to develop a potential therapeutic agents for FA as well as cancer with FA dysregulation.

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

POSTER #31

TONSL-FACT: ILLUMINATING UNSUSPECTED CULPRITS IN BREAST CANCER INITIATION

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Introduction: Immortalization overcomes the limited replicative potential of primary cells and is the earliest step in tumorigenesis. Due to a lack of sufficient primary cells, the genomic events associated with progression from immortalization to transformation have been studied extensively but not those leading to immortalization. Identifying immortalization-associated events is critical to develop early biomarkers and targets of tumorigenesis.

Methods: Breast epithelial cells from core biopsies of seven healthy women of diverse genetic ancestry were cultured and then immortalized by overexpression of human telomerase. RNA sequencing was done on the matched primary and immortalized cells, and EdgeR was used to perform differential expression analysis. Genes with significant expression changes during immortalization then underwent four additional filters to elucidate which have potential for tumorigenecity. First, genes were excluded if they were known to differ between progenitor and differentiated cells given already established distinctions in differentiation status between primary and immortalized cells. Next, evaluation via Ingenuity pathway analysis (IPA) was done on those with the largest fold changes to identify involvement in pertinent cancer and cell survival related networks. Finally, publicly available cancer transcriptome databases and CRISPR screens were used to identify breast cancer relevance and gene essentiality, respectively. The expression of selected genes was then measured across primary, immortalized, and RAS transformed cell lines using qPCR.

Results: Over 1,500 unique genes were differentially expressed between primary and immortalized cells, over 700 of which were upregulated in the latter. During IPA, many overlapped with pathways meaningful to tumorigenicity including cellular growth, proliferation, death, and survival. Of particular interest, TONSL (Tonsoku like, DNA Repair Protein) was found in multiple of these IPA pathways connected to the BRCA1 binding BARD1. Additionally, TONSL and the components of its interacting partner FACT (facilitates chromatin transcription) complex, SSRP1 and SUPT16H, were all listed as essential genes by CRISPR screening and were all significantly overexpressed in invasive breast cancer. Analyses of isogenic primary, immortalized and transformed cells confirmed expected upregulation in immortalized cells for these selected genes and demonstrated further upregulation in RAS transformed cells.

Conclusions: With the unique resource of primary breast cells from healthy women, the genomic event associated with immortalization and its link with tumorigenicity has been further explored. Based on the upregulation of TONSL and the FACT complex in immortalized cells, with further upregulation in RAS transformed cells, we propose that alterations in expression of genes involved in transcription, epigenome, and replication-associated DNA damage response are the earliest events in immortalization. These alterations could serve as tumor initiation biomarkers and potential therapeutic targets.

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

POSTER #32

TARGETING 17Q23 AMPLICON TO OVERCOME THE RESISTANCE TO ANTI-HER2 THERAPY IN HER2+ BREAST CANCER

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Chromosome 17q23 amplification occurs in ~11% of human breast cancers. Enriched in HER2+ breast cancers, the 17q23 amplification is significantly correlated with poor clinical outcomes. In addition to the previously identified oncogene WIP1, we uncover an oncogenic microRNA gene, MIR21, in a majority of the WIP1-containing 17q23 amplicons. The 17q23 amplification results in aberrant expression of WIP1 and miR-21, which not only promotes breast tumorigenesis, but also leads to resistance to anti-HER2 therapies. Inhibiting WIP1 and miR-21 selectively inhibits the proliferation, survival and tumorigenic potential of the HER2+ breast cancer cells harboring 17q23 amplification. To overcome the resistance of trastuzumab-based therapies in vivo, we develop pH-sensitive nanoparticles for specific codelivery of the WIP1 and miR-21 inhibitors into HER2+ breast tumors, leading to a profound reduction of tumor growth. These results demonstrate the great potential of the combined treatment of WIP1 and miR-21 inhibitors for the trastuzumab-resistant HER2+ breast cancers.

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

POSTER #33

GLIOBLASTOMA'S RESISTANCE TO IMMUNOTHERAPY IS MEDIATED BY A CD8+CD28- SENESCENT T CELL POPULATION

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Background: Glioblastoma (GBM) is the most common and aggressive primary central nervous system malignancy, with a mean survival time from diagnosis of 20.9 months even with aggressive treatment. The promise of immunotherapy has been tempered by GBM's remarkable level of immunosuppression, especially dysfunction of CD8+ T cells, the linchpin of most immunotherapies. Among the many facets of CD8+ T cell dysfunction, including tolerance, anergy, and exhaustion, CD8+ T cell senescence, as represented by the CD8+CD28- population, is observed in many cancers. However, the characteristics and function of CD8+CD28- T cells in GBM patients and their role in GBM induced immunosuppression and resistance to novel immune-based therapeutic agents remain unknown.

Hypothesis: CD8+CD28- T cells contribute to immunosuppression in GBM tumor microenvironment and ineffectiveness in CD8+ mediated tumor killing.

Methods: Blood and tumor samples from patients with GBM were collected at the time of surgical resection. Lymphocytes were isolated from tumor and blood. CD8+CD28- T cells from tumor and blood were analyzed by flow cytometry for various surface antigens and intracellular cytokines, and their characteristics were compared to their CD8+CD28+ counterpart. We also isolated lymphocytes from brain, blood, cervical lymph nodes, and spleen from mice bearing murine gliomas, and compared the CD8+CD28- and CD8+CD28+ T cells in their production of intracellular granzymes and other cytokines. We also investigated the telomere length of the CD8+ CD28- T cells in comparison to non-senescent T cells.

Results: Significant amount of CD8+CD28- senescent T cells are present in both the peripheral blood and tumor obtained at surgical resection. CD8+CD28- T cells are less efficient in mediating cytotoxic killing of cancer cells when compared to their CD8+CD28+ counterparts. This is demonstrated in both patients' samples and two murine models of glioma. In addition, CD8+CD28- senescent T cells are shown to express less inflammatory/regulatory cytokines, suggesting less functional competent when compared to CD8+CD28+ T cells. The phenotypical and functional changes in this CD8+CD28- T cells are telomere-independent senescence.

Conclusion:CD8+CD28- senescent T cells are a distinct population from exhausted CD8+ T cells and mediate further immunosuppression in GBM tumor microenvironment. The CD8+CD28-senescent T cells are not telomere-dependent replicative senescence but stress (cancer)-induced telomere-independent premature senescence. New treatment strategies aimed at depleting or reprogram these tumor-associated CD8+CD28- T cells and reverse T cell senescence are promising adjuvant therapy towards more effective GBM immunotherapy.

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

POSTER #34

METASTATIC COLORECTAL CANCER INDUCES MUSCULOSKELETAL AND METABOLIC ABNORMALITIES

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Background: Colorectal cancer (CRC) is a leading cause of death worldwide and in the most advanced state is often accompanied by the development of liver metastases and skeletal muscle wasting, *i.e.* cachexia. Despite affecting the majority of CRC patients, cachexia remains understudied and currently has no cure. A limited number of elementary characterized animal models for CRC are available, and only a single model of liver metastases associated with CRC has been developed for the study of cachexia. We aimed to further characterize this model by focusing on functional, molecular and metabolic effects on muscle. **Methods:** CD2F1 male mice were intrasplenically injected with C26 tumor cells (mC26) to mimic hepatic dissemination of cancer cells, while sham-operated animals received saline (n = 5/group). Animals were assessed weekly for body weight and grip strength. Upon sacrifice, tissues (muscles, liver, and bone) were collected for morphological and molecular analyses. **Results:** Liver metastatization by spreading of C26 cells was associated with progressive and significant loss of body weight (-13%; $p<0.0001$) in the tumor hosts. Consistently, mC26 bearers displayed significant reductions in muscle weights (gastrocnemius:-26%; $p<0.01$, tibialis anterior: -29%; $p<0.01$, quadriceps: -33%; $p<0.01$), supported by decreased muscle strength (-23%; $p<0.01$) and reduced muscle cross-sectional area (-22%; $p<0.05$). MicroCT analysis revealed that loss of skeletal muscle in mC26 hosts was accompanied by reductions in bone mass as indicated by reductions in trabecular bone volume fraction (BV/TV: -45%; $p<0.001$) and trabecular thickness (Tb.Th: -11%; $p<0.05$). At the molecular level, the skeletal muscle of mC26 mice showed reduced phosphorylation of the markers of protein anabolism mTOR, 4EBP1 and p70S6K, along with increased levels of phospho-STAT3, ubiquitin, MuRF-1 and Atrogin-1, thereby also suggesting enhanced protein catabolism. The muscle of mC26 hosts also showed prevalence of fibers with glycolytic metabolism and enhanced lipid accumulation, in line with mitochondrial abnormalities, as also evidenced by reduced levels of PGC1a and Mitofusin 2 and reduced enzymatic activity of succinate and pyruvate dehydrogenase. Metabolomics analysis by NMR revealed systemic reductions in glucose and reduced branched-chain amino acid levels, thus suggesting abnormalities in energy metabolism. **Conclusion**: Overall, our model recapitulates the cachectic phenotype of metastatic CRC and displays loss of muscle and bone mass, accompanied by reduced muscle anabolism, increased protein catabolism, abnormal mitochondrial homeostasis and metabolic deficits.

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

POSTER #35

PROMOTER METHYLATION STATUS OF A KEY METABOLIC ENZYME DICTATES CANCER CELL FATE WHEN NUTRIENT IS LIMITED

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Cancer cells strive to survive and thrive in nutrient-limiting environments by inducing gene expression that ensures adaption to sustain metabolic demands. During amino acid limitations, a master transcription factor ATF4 in the amino acid response pathway induces expression of asparagine synthetase (ASNS), which enables de novo asparagine biosynthesis. Administration of the enzyme L-asparaginase that depletes exogenous asparagine in blood stream is an important therapy option for acute lymphoblastic leukemia (ALL). However, ALL cells show sensitivity diversity to asparagine depletion and can gain resistance for therapy. Understanding the mechanisms regulating the cell endogenous *ASNS* expression is important to define the adaptive processes underlying cancer progression and treatment.

Here, we show that DNA hypermethylation at the *ASNS* promoter prevents its transcriptional expression following asparagine depletion. Insufficient expression of *ASNS* leads to asparagine deficiency, which facilitates an ATF4-independent induction of C/EBP homologous protein-CHOP (DDIT3/GADD153) that triggers apoptosis. These data suggest that chromatin accessibility is critical for ATF4 activity at the *ASNS* promoter, which can switch ALL cells from an ATF4-dependent adaptive response to ATF4-independent apoptosis during exogenous asparagine depletion. On the other hand, our patient-derived xenograft model data support that the upregulation of *ASNS* gene product in the bulk leukemia cells correlates with the promoter methylation status of *ASNS* gene which shifts from hyper- before L-asparaginase treatment to hypo- after L-asparaginase treatment, suggesting a mechanism of treatment resistance. Altogether, our results reveal a previously underappreciated epigenetic property for regulating a key metabolic enzyme gene for cancer cells' adaption to nutrient limitation, and warrant further investigation for cancer targeting strategies based on this finding.

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

POSTER #36

PHOSPHORYLATION OF HUMAN PAPILLOMAVIRUS E2 PROTEIN BY PROLINE-RICH TYROSINE KINASE 2 REGULATES VIRAL DNA REPLICATION

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High-risk HPV types cause approximately 5% of all cancers worldwide, with tumors of the cervix and oropharynx most common. The HPV E2 protein regulates several activities in the viral life cycle including viral gene expression, DNA replication, and mitotic partitioning of the viral genome. Apart from its interactions with other viral proteins, E2 interacts with host proteins that influence its function as well as other cellular processes. Post translational modifications of E2, specifically tyrosine phosphorylation, has been shown to regulate viral DNA replication.

The focal adhesion kinase PYK2 is a non-receptor tyrosine kinase that has been shown to be in complex with HPV18 E2 in proteomics studies. We show that PYK2 physically interacts with high risk HPV31 and HPV16 E2. Wild type (WT) as well as kinase-dead PYK2 co-immunoprecipitated E2 but only WT PYK2 was able to phosphorylate E2. Treatment of keratinocyte cell lines with PYK2 inhibitor PF-431396 resulted in enhanced viral DNA content. CRISPR-Cas9 mediated knockout of PYK2 increased viral DNA content in these cell lines. Co-immunoprecipitations of N-terminal truncated/deleted E2 proteins revealed that PYK2 interacted with and phosphorylated a region of amino acids 107-372 of HPV31 E2 protein. Using a series of tyrosine (Y) to non-phosphorylatable phenyl alanine (F) mutants of HPV31 E2, we show that specific Y residues are likely to be phosphorylated by PYK2 kinase.

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

POSTER #37

SESN3 DEFICIENCY PROMOTES CARCINOGEN-INDUCED HEPATOCELLULAR CARCINOMA VIA REGULATION OF THE HEDGEHOG PATHWAY

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Sestrin 3 (Sesn3) belongs to a small sestrin protein family that has been implicated in multiple biological processes including anti-oxidative stress, anti-aging, cell signaling, and metabolic homeostasis. However, the role of Sesn3 in hepatocellular carcinoma (HCC) remains unclear. In this study, we generated a Sesn3 knockout (KO) mouse model and induced the HCC development by combination of a single dose of diethylnitrosamine (DEN) and chronic feeding of a choline deficient-high fat diet (CD-HFD). After 6 months of the dietary treatment, Sesn3 KO mice developed more severe HCC with higher levels of alpha-fetoprotein, arginase 1, and CK-19, but also higher metastatic rates than the WT mice. Histological analysis revealed elevated extracellular matrix and cancer stem cell markers including Acta2, CD44, and CD133. Signaling analysis identified elevated IL6-Stat3 and Akt pathways. Biochemical and microscopic analysis uncovered a novel inhibitory regulation of Gli2, a downstream of the hedgehog signaling, by Sesn3. Two of the Gli2-regulated genes – Pdgfrb and CD44 were upregulated in the Sesn3-deficient liver tissue or cells. In conclusion, our data suggest that Sesn3 plays a critical tumor suppressor role in the liver partly through the inhibition of the hedgehog signaling.

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

POSTER #38

MODELING CELLULAR PLIANCY-DEPENDENT VARIABILITY IN ONCOGENE-INDUCED TRANSFORMATION AND THERAPEUTIC RESPONSE IN BREAST CANCER

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Breast cancers have been classified into multiple intrinsic subtypes. The contributions of cell-of-origin, inter-individual variability in pliancy of breast epithelial cells and cancer-specific genomic aberrations in genesis of specific subtypes and therapeutic response of breast cancer are unknown. We took advantage of our recently developed model system of immortalized breast epithelial cell lines derived from healthy breast tissue of women of different genetic ancestry to address this issue. Immortalized cell line with gene expression patterns similar to luminal A subtype (KTB34 from European ancestry), normal-like subtype (KTB39 from African ancestry) and basal subtype (KTB22 from Latina ancestry) were transformed using defined oncogenes H-RasG12V and SV40-T/t antigens. Signaling pathways downstream of these oncogenes are relevant for breast cancer, as activation of RAS signaling pathway is frequently observed in endocrine therapy resistant breast cancers, whereas SV40-T/t antigens inactivates tumor suppressors retinoblastoma and p53. Retinoblastoma and p53 inactivation are common in luminal type B and triple negative breast cancers, respectively. Flow cytometry, single cell genomics and tumorigenesis in NSG mice were used to characterize immortalized and transformed cells. Immunofluorescence staining with Phospho-gH2A.X and RAD51 antibodies as well as Annexin V staining was used to measure response to chemotherapeutic drug doxorubicin. SV40-T/t antigens overexpression but not mutant Ras overexpression had cell line specific effects on stem/progenitor/mature cell hierarchy of the transformed cells. Single cell analyses of paired immortalized and transformed cells showed remarkable variability in transformation-prone cell population between cell lines. While almost all the nine subpopulation of cells in the luminal A cell line were prone for transformation, select subpopulation of cells within normal-like (5 out of 10) and basal (4 out of 12) immortalized cell lines were prone for transformation. In vivo studies demonstrated that while luminal A cell line generated four distinct types of tumors that differentially expressed luminal cell markers such as FOXA1 and GATA3, normal-like and basal cell lines generated cytokeratin 14 positive basal tumors with squamous carcinoma features. SV40-T/t antigens transformed but not Ras-transformed cells were more sensitive to doxorubicin-induced DNA damage and were impaired in repairing damaged DNA. These results suggest that while cell-of-origin and cellular pliancy determine the molecular subtype of breast cancer, oncogenic aberrations determine stem/progenitor hierarchy, consequently tumor heterogeneity and response to DNA damage-inducing chemotherapeutic drugs.

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

POSTER #39

COLLABORATIVE REGULATION OF KERATINOCYTE GROWTH, LONGEVITY, AND DIFFERENTIATION BY HPV TYPE 16 E6 AND NFX1-123

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High-risk human papillomaviruses (HR HPV) are the causative agent of multiple anogenital and oropharyngeal cancers, accounting for approximately 5% of the global burden of cancer. Because HR HPVs require both epithelial cell proliferation and differentiation for the viral life cycle, they manipulate the cell to drive these processes and in doing so, secondarily support oncogenesis. To achieve these functions, HR HPV proteins must partner with and often co-opt cellular proteins. We have previously demonstrated a partnership between HR HPV type 16 E6 (16E6) and NFX1-123, an endogenous epithelial cell protein. This partnership collaboratively increases expression of telomerase and levels of Notch1, a master regulator of cellular growth and differentiation. Although normal functions of NFX1-123 in the epithelium are not well described, its levels are greatly increased in cervical cancer, suggesting its importance in the course of long term HPV infection or HPV-associated oncogenesis.

We identify for the first time a role for NFX1-123 in regulating keratinocyte differentiation and late events of the HPV life cycle. NFX1-123 itself increased with differentiation of epithelial cells. Greater NFX1-123 augmented differentiation marker expression and JNK phosphorylation in HPV16-positive W12E cells. This was associated with increased expression of MKK4 and MKK7, upstream kinase regulators of JNK phosphorylation. Crucially, levels of NFX1-123 also correlated with expression of L1, the capsid protein of HPV that is only expressed during epithelial cell differentiation. Altogether, these studies define a role for NFX1-123 in potentially linking expression of cellular genes and HPV genes during differentiation.

Interestingly, 16E6 and NFX1-123 were able to augment not only epithelial differentiation, but growth and proliferation of keratinocytes as well, despite these pathways typically being mutually exclusive. 16E6 and NFX1-123 have previously been shown to target the known oncogenic protein telomerase by increasing expression and activity of its catalytic subunit, hTERT. Over long term cell culture, keratinocytes expressing 16E6 and overexpressing NFX1-123 had extended active growth, decreased senescence marker staining, and more rapid cell cycling compared to 16E6 expressing cells with endogenous amounts of NFX1-123. These effects on cell proliferation were associated with increased telomerase activity and expression of hTERT. HPV 16-positive cervical cancer cell lines with knocked down NFX1-123 had slowed growth and reduced hTERT. Altogether HR HPV type 16 E6 and NFX1-123 modify multiple epithelial cell pathways, which subsequently alter the host cell milieu to potentially promote HPV infection and support oncogenic development.

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

POSTER #40

TARGETING TRIPLE NEGATIVE BREAST CANCER WITH PRECISION NANOBOMB

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Triple negative breast cancer (TNBC), which accounts for 15-20% of all breast cancer, is highly aggressive and metastatic. TNBC has the highest death rate in the first two years and the poorest overall survival of all breast cancer types. Despite the fact that targeted therapies have become the first-line treatment for many different types of cancers, treatment options for TNBC are limited and associated with severe toxicities. Notably, 50% of TNBC patients exhibit hemizygous deletion of *TP53*, and this frequent genomic event is significantly associated with poor survival of TNBC patients. Through *in silico* analysis, we identified *POLR2A* in the *TP53*-neighboring region as a collateral vulnerability target in TNBC tumors, suggesting that its inhibition via small interfering RNA (siRNA) may be an amenable approach for TNBC targeted treatment. To enhance bioavailability and improve endo/lysosomal escape of siRNA, we designed pH-activated nanoparticles for augmented cytosolic delivery of POLR2A siRNA (siPol2). Suppression of POLR2A expression with the siPol2-laden nanoparticles (siPol2@NPs) leads to enhanced growth reduction of tumors characterized by hemizygous POLR2A loss. These results demonstrate the potential of the pH-responsive nanoparticle and the precise POLR2A targeted therapy in TNBC harboring the common *TP53* genomic alteration.

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

POSTER #41

XRN2 DEPLETION IS SYNTHETIC LETHAL WITH PARP1 INHIBITION

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Persistent R-loops (RNA-DNA hybrids with a displaced single-stranded DNA) create DNA damage and lead to genomic instability. The 5'-3'-exoribonuclease 2 (XRN2) degrades RNA to resolve R-loops and promotes transcription termination. Previously, XRN2 was implicated in DNA double strand break (DSB) repair and in resolving replication stress. Here, using tandem affinity purification-mass spectrometry, bioinformatics, and biochemical approaches, we found that XRN2 associates with proteins involved in DNA repair/replication (Ku70-Ku80, DNA-PKcs, PARP1, MCM2-7, PCNA, RPA1), and RNA metabolism (RNA helicases, PRP19, p54(nrb), splicing factors). Novel major pathways linked to XRN2 include cell cycle control of chromosomal replication and DSB repair by non-homologous end joining. Investigating the biological implications of these interactions led us to discover that XRN2 depletion compromised cell survival after additional knockdown of specific DNA repair proteins, including PARP1. XRN2-deficient cells also showed enhanced PARP1 activity. Consistent with concurrent depletion of XRN2 and PARP1 promoting cell death, XRN2-deficient fibroblast and lung cancer cells also demonstrated sensitivity to PARP1 inhibition. The XRN2 alterations (mutations, copy number/expression changes) are frequent in cancers. Thus, PARP1 inhibition could target cancers exhibiting XRN2 functional loss. Collectively, our data suggest XRN2's association with novel protein partners and unravel synthetic lethality between XRN2 loss and PARP1 inhibition.

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

POSTER #42

EFFECTS OF RANKL PRODUCING COMPARED TO NON-RANKL PRODUCING TUMORS ON MUSCLE AND BONE

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Non-bone tumors can have dramatic effects on bone mass even in the absence of metastases. Many of these tumors induce skeletal muscle loss resulting in cachexia. To determine if cachexia and bone loss could be linked, two cancer models were examined, the ES-2 high-grade serous ovarian cancer and the C26 adenocarcinoma. As previously shown, ES-2 tumor growth was associated with body wasting, muscle atrophy and bone loss (Pin et al, JCSM 2018). The C26 model was also characterized by body and muscle wasting (Bonetto et al, JVE 2016), but here we show only a modest effect on bone mass (Tb.Th. -10% *vs.* C,$p<0.05$). In accordance, bone histomorphometry showed a dramatic increase in osteoclasts in the femurs from ES-2 hosts (OcS/Bs +149%, $p<0.01$; N.Oc/B.Pm +37%, $p<0.05$) and, though a trend, no significant effects were observed in the C26-bearing mice. Both models showed a dramatic increase in osteocyte apoptosis (+2800%, $p<0.001$ for ES2; +2500% $p<0.001$ for C26) and empty lacunae (+93%, $p<0.05$ for ES2; +100%, $p<0.05$ for C26). On the other hand, expression of sclerostin was downregulated in the bone from C26 tumor bearing mice (-90% *vs.* C, $p<0.001$), but no change was detected in osteocyte sclerostin in the ES-2 hosts. To identify the cause of the dramatic bone resorption, RANKL was found increased in the plasma of ES-2 mice (+420% *vs.* C, $p<0.05$), but decreased in the C26 hosts (-70% *vs.* C, $p<0.05$). Interestingly, the ES-2 cells were found to be a source of RANKL, as shown by high levels in the ascites of the ES-2 mice (140 ¿g/ml) and highly elevated secretion by cultured ES-2 cells (+246%, $p<0.001$) compared to the C26. In order to investigate whether elevated RANKL could play a role not only in bone loss, but also in muscle wasting, we exposed mature C2C12 myotubes to 200 ng/ml recombinant RANKL. After 72 hours of treatment the myotubes exhibited marked fiber atrophy (-20% *vs.* C, $p<0.01$), also consistent with elevated TRAF6 and reduced activation of the anabolic AKT/mTOR pathway, thus suggesting that RANKL might be responsible for muscle wasting in the ES2 mice by directly impairing muscle protein anabolism. Overall, these studies emphasize that non-metastatic tumors can have differential effects on bone and suggest that treatment should be adjusted to target tumor type. Studies are underway to determine if anti-RANKL therapies may serve as strategies to reduce the negative effects associated with growth of the ES-2 tumor not only on bone, but also on muscle.

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

POSTER #43

ALLELE-SPECIFIC EXPRESSION AND HIGH-THROUGHPUT REPORTER ASSAY REVEAL FUNCTIONAL VARIANTS IN HUMAN BRAINS WITH ALCOHOL USE DISORDERS

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Genome-wide association studies (GWAS) of complex traits such as alcohol use disorders (AUDs) usually identify variants in non-coding regions, and cannot by themselves distinguish whether the associated variants are functional or in linkage disequilibrium with the functional variants. Transcriptome studies can identify genes whose expression differs between alcoholics and controls. To test which variants associated with AUDs may cause expression differences, we integrated data from deep RNA-seq and GWAS of four postmortem brain regions from 30 subjects with AUDs and 30 controls, and analyzed allele-specific expression (ASE). We identified 90 genes with differential ASE in subjects with AUDs compared to controls. Next, to test one potential mechanism of action of those genes, we studied single nucleotide polymorphisms (SNPs) in the 3' untranslated regions (3'UTR) of the genes identified in the ASE analysis. Of the 90 genes with differential ASE, 61 genes contained 437 SNPs in the 3'UTR with at least one heterozygote among the subjects studied. Using a modified PASSPORT-seq (parallel assessment of polymorphisms in miRNA target-sites by sequencing) assay, we identified 25 SNPs that affected RNA levels in a consistent manner in two neuroblastoma cell lines, SH-SY5Y and SK-N-BE(2). Many of these are in binding sites of miRNAs and RNA binding proteins, indicating that these SNPs are likely causal variants of AUD-associated differential ASE. In sum, we introduce a combination of computational and experimental approaches and provide a powerful strategy to uncover functionally relevant variants associated with the risk for AUDs.

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

POSTER #44

BONE-DERIVED TGF-ß IMPAIRS GLUCOSE METABOLISM AND INSULIN RELEASE BY OXIDATION OF RYR2 CA2+ RELEASE CHANNEL IN PANCREATIC Ss-CELLS IN THE SETTING OF HIGH BONE TURNOVER, AGING AND HIGH FAT DIET

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Bone destruction in cancer or other pathology causes fractures, pain and muscle weakness.TGF-ß released from bone via osteoclastic bone resorption, acts systemically to cause skeletal muscle weakness through oxidation of sarcoplasmic reticulum Ca^{+2}channel, ryanodine receptor (RyR). Since oxidation of pancreatic-b-cell RyR2 can impair insulin secretion and bone destruction results in systemic TGF-ß effects, we hypothesized thatstates of increased bone resorption with release of TGF-ßcauses oxidation of pancreatic-ß-cell RyR2 to impair insulin secretion and glucose homeostasis.

We studied the effects of bone-derived TGF-b on the pancreas using a model of Camurati-Engelmann disease (CED), a bone dysplasia with increased bone turnover. CED mice had increasedcirculating TGF-ß, reduced serum insulin, and increased pSmad2/3in pancreatic-b-cells. Forty-five-week-oldCED mice fed high-fat diet (HFD) for 15 weeks developed glucose intolerance ($p<0.01$), and impaired insulin ($p<0.01$) secretion (glucose-stimulated insulin secretion in isolated islets) vs HFD-WT mice. Both HFD-CED and HFD-WT had insulin resistance (via ITT) compared with CED and WT fed low-fat diet (LFD). HFD-CED mice had higher fat mass ($p<0.001$), skeletal muscle weakness ($p<0.001$), and reduced muscle-fiber diameter ($p<0.001$) compared to HFD-WT.HFD-CED mice had reduced bone mineral density ($p<0.001$)and increased cortical porosity ($p<0.01$) compared to HFD-WT mice. Impaired insulin secretion and skeletal muscle weakness in HFD-CED mice were associated with Nox4-mediated oxidation of pancreatic ß-cellRyR2 and skeletal muscle RyR1 respectively. TGF-ß had direct effects on insulin secretion as isolated pancreatic islets from WT mice treated with TGF-ß showed increased phosphoSmad3 and Nox4-mediated oxidation of RyR2. Further,TGF-ßdecreased expression of pro-insulin (*ins-1 and ins-2*) mRNA

Collectively, these data suggest that states of increased bone destruction can disrupt glucose metabolism, pancreatic ß cell insulin secretion and causes muscle weakness, via systemic effects of bone-derived TGF-ß to oxidize RyR. These effects, exacerbated by HFD and aging, have implications for bone health as impaired glucose metabolism and muscle weakness can further increase fracture risk. Blocking bone destruction, the release of TGF-ß and preventing RyR Ca^{2+}leak in pathologic bone destruction should reduce fracture risk by improving hyperglycemia, muscle weakness and subsequent bone quality.

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

POSTER #45

SPONTANEOUS OVARIAN ENDOMETRIOSIS IN A MOUSE MODEL WITH DELETION OF ARID1A AND GAIN OF ONCOGENIC KRAS

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Endometriosis is an estrogen-responsive disease characterized by growth of endometrial-like tissue outside the uterus. Endometriosis affects roughly 10% of US women. Endometriosis is a significant risk factor for ovarian cancer, placing 5 million US women at 2-3 fold-increased risk of endometriosis-associated ovarian cancers. Work has shown that loss of function mutations in ARID1A (AT-rich interactive domain 1A) are frequent in endometriosis-associated ovarian cancers. Additionally, ovarian cancers from women with concurrent endometriosis are molecularly distinct, with activation of KRAS signaling. We have created a genetically engineered mouse model of spontaneous ovarian endometriosis by deleting *Arid1a* and adding oncogenic *Kras* to the ovaries (*Arid1a*$^{flox/flox}$;*Kras*^{G12D}; *Amhr2*$^{Cre/+}$, AKC mice). As early as 6 weeks, AKC mice develop abnormal follicles; characterized by multiple oocytes, oocytes stuck in the corpus luteum, non-centralized oocytes, or broken and ring-shaped zona pellucida. By 8 weeks, AKC mice develop gross lesions consistent with blood filled abnormal follicles and epithelial lined cysts. Interestingly, the histological phenotype of epithelial lined cysts and gross surface lesions can be seen as early as 3 weeks when AKC mice are stimulated with gonadotropins. Preliminary data suggests the histological phenotype of epithelial lined cysts and gross surface lesions is estrogen sensitive and progesterone insensitive as witnessed by implanting either E2 (estradiol) or P4 (progesterone) pellets into 6 week old mice and observing gross lesions at 12 weeks. Older unstimulated mice develop frank endometriosis, as evidenced by hemosiderin-laden macrophages, endometrial glands, and endometrial stroma as early as 9 months. AKC female mice develop cancers in the lung, vagina-skin junction, and the ovary. Ovarian malignant spindle cell carcinoma developed in 22% (5/23) with concurrent endometriosis in the same ovary. In addition to altered hormone and gonadotropin responses, preliminary data suggests that AKC mice have an abnormal immune response with elevated T-regulatory and T-helper cell populations in the spleen and lymph nodes. Thus, modulating the immune system or gonadotrophic hormones in AKC female mice may lead to a better understanding of the development and progression of endometriosis and endometriosis-associated ovarian cancers.

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

POSTER #46

ROLE OF THE TWO ISOFORMS OF ST2, THE IL-33 RECEPTOR, IN ACUTE GRAFT-VERSUS-HOST DISEASE

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Acute graft-versus-host disease (aGVHD) remains a devastating complication after allogeneic hematopoietic cell transplantation (HCT). We previously identified high plasma soluble STimulation-2 (sST2), the decoy form of IL-33 receptor, as a biomarker of aGVHD and death. sST2 sequesters IL-33, limiting its availability to T cells expressing the transmembrane molecule form of ST2 (ST2) [mostly T helper 2 (Th2) cells and $ST2^{+}FoxP3^{+}$ regulatory T cells (Tregs)]. Furthermore, blockade of the full ST2 in the peritransplant period with a neutralizing anti-ST2 monoclonal antibody prevents severe aGVHD and its related mortality. To further define the contribution of the two isoforms in the pathogenesis of aGVHD, we generated a knockin of the membrane ST2 intron 8-9 in place of sST2 exon 8 by using CRISPRCas9 technology. In this way, only membrane ST2 can be expressed and no sST2 can be generated. We called this mouse $sST2^{-/-}$. We confirmed on sorted T cells isolated from naïve $sST2^{-/-}$ mice that conventional T cells (Tcons) did not express sST2, and that membrane ST2 expression on T cons, particularly Tregs was significantly increased in $sST2^{-/-}$ mice ($p<0.0033$ for total T cell, $p<0.0032$ for Tregs) compared to WT mice. This translates to the fact that there are more ST2-expressing Tregs in the donor graft. We, next, hypothesized that transplantation of donor $sST2^{-/-}$ T cells will prevent aGVHD development and mortality better than the full $ST2^{-/-}$ T cells.Surprisingly, transplantation of donor $sST2^{-/-}$ T cells induced similar aGVHD symptoms and survival. Although the graft contained more $ST2^{+}$ Tregs, analysis of T cells in the intestine (the main aGVHD target organ) at day 10 post-HCT showed frequencies of Tregs and $ST2^{+}$Tregs were similar and decreased, respectively, in recipients who received $sST2^{-/-}$ donor T cells compared to WT. As we have shown that Tcons were the main source of sST2 in aGVHD, we further hypothesize that transplantation of donor $sST2^{-/-}$ Tcons with WT Tregs will prevent aGVHD while transplantation of donor WT Tcons with $sST2^{-/-}$ Tregs will lead to outcomes similar or worse than transplantation with $sST2^{-/-}$ total T cells. In addition, the mechanism we suggest is through $ST2^{+}$ Tregs exhaustion during homeostatic proliferation. We conclude that transplantation with donor $sST2^{-/-}$ total T cells does not reproduce the protective aGVHD effect of transplantation with full $ST2^{-/-}$ total T cells possibly due to the different roles of the two isoforms of ST2.

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

POSTER #47

KUB5-HERARPRD1B REGULATES CDK1 EXPRESSION THROUGH THE DREAM COMPLEX

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Triple Negative Breast Cancer (TNBC) is a subtype with more aggressive, less differentiated capacity and enhanced metastatic ability. Although TNBC tumors are more responsive to neoadjuvant chemotherapy compared to receptor-positive tumors, development of resistance to cytotoxic chemotherapy and early relapse is more common. Due to the lack of effective therapies, understanding the underlying mechanisms of TNBC aggressiveness and poor prognosis is a major focus of research with particular interest in identifying new therapeutic targets.

Kub5-Hera^{RPRD1B}(K-H) is a novel factor involved in preserving genomic stability and regulates BRCA1 function by mediating the expression of CDK1, a master regulator of cell cycle, DNA replication, and DNA repair. This regulation has been reported in MDA-MB-231 cells, a model of TNBC. However, the underlying mechanism regulated CDK1 expression by K-H is unknown. Here, we found that the CDK1 promoter is essential for the recruitment of K-H. We also examined the interaction between K-H and the complex for the regulation of cell cycle-dependent gene expression, DREAM complex (dimerization partner, RB-like, E2F and multi-vulval class B complex) by immunoprecipitation. The interaction between K-H and the DREAM complex is further validated using a Proximity ligation assay (PLA). Furthermore, a mutation in K-H (R106A) results in the loss of an interaction between K-H and the DREAM complex as well as K-H and RNAPII, leading to decreased CDK1 levels. These results suggest that CDK1 expression is upregulated by K-H and its complex formation with the DREAM complex and RNAPII. Thus, we hypothesize that K-H modulates CDK1 expression through the recruitment of the DREAM complex to the CDK1promoter region in a sequence-specific manner. Our studies reveal a novel strategy for developing therapies that target the interaction between K-H and the DREAM complex, potentially inhibiting the growth of cancer cells. *This work is supported by NIH grant* ***1 R01CA201489-04.***

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

POSTER #48

GENOMIC CORRELATION OF QUANTIFIED EPITHELIAL AND STROMAL TISSUES ON HISTOPATHOLOGICAL IMAGES

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The epithelial and stromal tissues are integral parts of tumor microenvironment (TME) and are pivotal for understanding tumor initiation and progression. Distinguishing stroma from epithelial tissues is critically important for quantitative characterization of TME, especially for cancer histological image analysis. Manual examination and assessment of these tissue regions is time-consuming and often infeasible for large-scale studies. Modern automatic image analysis procedures are highly desired to reduce labor cost and provide effective alternatives in diagnostic decision-making. Taking advantage of deep learning methods which have shown outstanding performance in image processing tasks, we propose an automatic pipeline to identify epithelial and stromal tissues in H&E stained whole-slide images. We present spatial quantification and genomic correlation of both the tissues for three breast cancer subtypes: ER positive, ER negative and triple negative, leading to which genes contribute to development of different tissues in various subtypes. We found that tissue components in pathological images vary widely for different breast cancer subtypes. Gene Ontology enrichment results suggest the same tissue is associated with similar biological processes in different subtypes, while each subtype has their own idiosyncratic biology processes governing the development of these tissues. For the genes correlated with the epithelial tissues, they are enriched in biology processes for cell cycle, among which sister chromatid segregation, nuclear division, and mitotic cell cycle are the most commonly enriched GO terms shared by the three breast cancer subtypes. However, we also observed specifically enriched GO terms and genes for each subtype that corresponds to different cell cycle stages. The Growth phase related genes including G1 phase and G2 phase are specifically enriched for ER positive subtype, whereas Mitotic phase genes are specifically enriched for the triple negative subtype and S phase related genes are specific for ER negative subtype. Similarly, such patterns of shared high-level biological processes with specific functions are also observed for the stromal tissues. For the stromal tissue, the most significantly enriched GO biological process terms are all related to the development of TME including vasculature development, cellular component movement, and growth factor stimuli related GO functions which are shared among the three breast cancer subtypes. For the ER positive subtype, angiogenesis related genes are specific enriched, while for triple negative subtype, muscle structure genes (especially the ones related to actin fibers and cytoskeleton) are specifically enriched. In addition, for the ER negative subtype, growth factor genes are enriched. These results are consistent with underlying biological processes for cancer development, which further affirms the robustness of our image processing method. Taken all together, our study can bring new insights into the development of whole-slide imaging based genomic and biological processes for various types of cancers.

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

POSTER #49

PROTEIN INTERACTIONS BETWEEN MUTANT P53 AND ETS TRANSCRIPTION FACTORS

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p53 has tumor suppressive functions that are frequently inactivated in many types of cancer by a single nucleotide missense mutation. In addition to the well-studied tumor suppressive functions, recent findings indicate that this mutant p53 (mp53) can promote tumor growth through a gain-of-function activity. It is thought that gain of function activity is mediated by mp53 interaction with the ETS family transcription factor ETS2, which brings mp53 to DNA where it can act as a co-activator. The ETS family of transcription factors have 28 members which all share a similar DNA binding domain. It is unclear if mp53 function is dependent on only ETS2, or if it can bind other ETS family transcription factors. To test the specificity of mp53 across the ETS family, we used a panel of purified ETS proteins. These ETS proteins were used to pull-down mp53(R248W) from the cell lysate of prostate cancer cell line VCaP or purified mp53(R248W). The results showed that the ETS family transcription factors ETS1, ETS2, ETV4, SPI1, and ERG had strong interactions with both mp53 in VCaP cell lysate and purified mp53, while ETV6 and FLI1 had strong interactions with purified mp53 but no interaction with mp53 in VCaP cell lysate. In conclusion, mp53 may promote tumorigenesis through the gain-of-function that can interact with a variety of ETS family transcription factors, and this may vary based on cell type.

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

POSTER #50

A PRECLINICAL MOUSE MODEL OF CLEAR CELL ENDOMETRIAL CARCINOMA

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Endometrial cancer is the most frequent gynecologic malignancy, with 61,880 new cases diagnosed in 2019 in the US. While women with early stage disease are routinely cured with simple hysterectomy, the prognosis for women with aggressive forms of the disease (*i.e.,* high-grade papillary serous carcinoma, clear-cell carcinoma, or uterine carcinosarcoma) is dismal. These aggressive endometrial cancers account for only 15% of tumors, but they account for 50% of deaths. Hindering improvement of treatment is the lack of preclinical mouse models of these aggressive carcinomas. Recent evidence suggests that mutations in *DICER*, the ribonuclease III required for miRNA synthesis, are frequent in endometrial cancer. Importantly, 50% of *PTEN* mutant clear cell endometrial carcinomas also contain mutations in *DICER*. To study the role of DICER in endometrial cancer, we deleted *Dicer* in a mouse model of endometrial cancer $Pgr^{Cre};Pten^{f/f};Dicer^{f/f}$ (dcKO). Our dcKO mice had decreased survival due to endometrial cancer. Compared with control uteri, dcKO uteri had similar weight but grossly had areas of solid and cystic lesions interspersed across the uterine horns. As early as 3 weeks, dcKO uteri exhibited low-grade endometrioid carcinoma. By 12 weeks, dcKO uteri exhibited a mixture of aggressive high-risk histologies including high-grade endometrioid, high-grade clear cell, and uterine carcinosarcoma. As early as 8 weeks, dcKO mice exhibited metastatic disease in the oviduct and ovaries. Removal of ovarian hormones resulted in smaller dcKO uteri but with increased penetrance of high-grade clear cell adenocarcinoma. Global transcriptomic analysis revealed 1892 unique mRNA and 51 mature miRNA molecules dysregulated in dcKO compared to control uteri at 3 weeks (log 2 fold change >1 or <-1, $P<0.05$). Examination of steroid hormone responsive genes revealed baseline increased expression of estradiol-responsive genes and decreased expression of progesterone-responsive genes. Our *in vivo* model system represents an important tool to develop novel therapy for clear cell endometrial carcinoma.

Basic Science ***Research Associate***

POSTER #51

THE EFFECTS OF COMBINED TREATMENT OF DNMT/EZH2 INHIBITORS AND ANTI-PD-L1 IN THE IMMUNE CELL LANDSCAPE OF A PRE-CLINICAL MOUSE MODEL OF GLIOBLASTOMA

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Background: Glioblastoma (GBM) is an aggressive brain tumor with poor prognosis and is notoriously known for its immunosuppressive tumor microenvironment (TME) and infiltrative growth. Factors that contribute to GBM's immune suppression include preferential T-regulatory cell (Tregs) infiltration, absence of well-orchestrated anti-GBM immune response, poor tumor antigenicity, and high expression of immune checkpoint receptors, like PD-L1. Th1 cells are known for their anti-tumor response via IFN-¿ production and are attracted by the chemokines IL-16, CXCL9 and CXCL10. Immunotherapy is an exciting avenue of GBM treatment since the immune system can cross the BBB and eliminate tumor cells without harming eloquent brain tissue. DNMT and EZH2 are known methylators of DNA and histones, respectively, and their inhibitions have shown to slow tumor growth in many cancers, such as AML or colon cancer. In addition to their epigenetic functions, epigenetic modifiers are also known to have immune modulating functions. DNMT and EZH2 inhibitors are currently being tested in clinical trials as tumorisidal agents for solid tumors, however their potential as immune modifiers is not well characterized. Combining these inhibitors alongside anti-PD-L1 could prove a viable treatment option for GBM patients and improve survival.

Hypothesis: We hypothesize that inhibition of DNMT and EZH2 alongside treatment with anti-PD-L1 will modify immune cell infiltrate of the TME by increasing Th1 populations via blockade of PD-L1 and increase IL-16, CXCL9 or CXCL10 production.

Methods: C57BL/6 mice were intracranially injected with 400,000 GL261 tumor cells in PBS. GL261-injected mice were randomly placed into four groups and were injected intraperitoneally with either PBS, 5-aza-dc/EPZ-6438, anti-PD-L1 antibody, or a combination of 5-aza-dc, EPZ-6438 and anti-PD-L1 antibody (combination group.) The mice were euthanized one week post-operation (wpo) and lymphocytes were isolated from brain, cervical lymph nodes, peripheral blood, and spleen. Lymphocytes were then stained with CD3, CD4, T-Bet, GATA3, FoxP3, and ROR¿t and analyzed with flow cytometry. Brain tumor was conserved post-lymphocyte isolation and protein lysate was obtained for Western blot analysis. Brain tumor lysate was probed for IL-16, CXCL9, and CXCL10 with Western blotting.

Results: GL261-injected mice in the combination group showed an increase Th1 population one wpo as compared to the other groups. Western blot analysis of brain tumor post-lymphocyte isolation showed increased CXCL9 expression as compared to the other treatment groups but not to the PBS group.

Conclusions: Relief of immunosuppression and treatment with epigenetic modifiers allowed a Th1 cell population to thrive in the TME via blockade of the PD-1/PD-L1 axis and CXCL9 production. Increase Th1 populations indicate a more active and immunogenic anti-tumor response. Future studies include survival studies with the treatment options and testing out other treatment combinations, such as chemotherapy.

Basic Science ***Research Technician***

POSTER #52

LIPPIA ORIGANOIDES EXTRACT INHIBITS MITOCHONDRIAL METABOLISM AND INDUCES A PERMANENT DECREASE IN VIABILITY OF MDA-MB-231 TRIPLE NEGATIVE BREAST CANCER CELLS

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Breast cancer is the most common type of cancer worldwide in women, affecting approximately 1 in 8 females in the U.S. The most aggressive subtype of breast cancer, triple negative breast cancer (TNBC), is typically resistant to conventional therapies that target hormone receptors. Previous studies have shown that a supercritical fluid extract from the plant Lippia origanoides (L42) possesses significant anti-cancer properties. In this study we confirm the concentration-dependent decrease in viability of MDA-MB- 231 cells treated with L42, as well as a significant increase in cleaved caspase 8 in MDA-MB-231 cells treated with L42, demonstrating the activation of the extrinsic pathway of apoptosis, and thereby explaining the decrease in cell viability. We also show that the mitochondrial metabolic pathways were targeted by the extract. In this study we provide evidence that L42 is a potential source of bioactive compounds that could be used for alternative treatment for TNBC.

Basic Science ***Undergraduate Student***

POSTER #53

EPISODIC MEMORY ACTIVATION PRIOR TO SYSTEMIC TREATMENT IN OLDER BREAST CANCER PATIENTS

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Background: Lower than expected cognitive functioning has been reported in breast cancer patients prior to systemic treatment. The literature has primarily focused on younger breast cancer patients, and there have been few pretreatment neuroimaging studies. Older patients may be at higher risk for cancer-related cognitive declines due to aging processes. We used fMRI to examine brain activation patterns during episodic memory encoding in older breast cancer patients prior to systemic treatment.

Methods: Sixteen patients (mean age 68.3 years, mean education 15.3 years) studied post-surgery but prior to systemic therapy and 16 matched healthy controls (mean age 68.2 years, mean education 15.6 years) were recruited at a single site as part of a larger multi-site longitudinal cognitive study. All participants completed a blocked-design episodic memory scene encoding fMRI task. Encoding accuracy was assessed with an out-of-scanner recognition test. Between-group differences in brain activation and correlations between activation and task performance were analyzed using SPM12 and SPSS, respectively.

Results: Task performance accuracy and reaction time did not differ between patients and controls. During scene encoding, patients demonstrated significantly ($p<0.01$) reduced activation in left parahippocampal, insular, and precuneus regions relative to controls. Left parahippocampal activation was significantly ($p<0.05$) positively correlated with task performance across the whole cohort and in patients alone (higher parahippocampal activation was seen with more accurate recognition of new stimuli).

Conclusions: These results are consistent with the limited neuroimaging literature demonstrating altered brain function prior to systemic treatment for non-CNS cancer, and extend the previous work by examining episodic memory processing in older patients. Data acquisition to increase the cohort size is ongoing, and participants will be followed longitudinally to examine the course of these pre-treatment functional alterations post-treatment, to advance understanding of the relationship between cognitive effects of cancer and treatment and risk for age-related cognitive declines.

Behavioral ***Faculty***

POSTER #54

PREDICTORS OF DEATH FOR PATIENTS TREATED WITH PALLIATIVE INTENT RADIATION USING PROSPECTIVE DATABASES

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Background: Radiation therapy (RT) effectively palliates pain and other symptoms due to cancer and is frequently offered to patients with metastatic disease. Standard palliative RT schedules range from one to =10 fractions; however, prognostic variables related to survival following palliative RT are not well studied. A better understanding of prognostic factors in this setting will assist in selecting appropriate palliative RT regimens for patients with short life expectancies.

Methods: Prospectively maintained institutional databases tracking morbidity and mortality and outcomes of an institutional peer review process were used to identify patients who received palliative RT between April 2015 - December 2018. Cox proportional hazards model was used to identify factors correlated with overall survival (OS) and mortality within 30 days (30DM) after completing palliative RT, including age, sex, admission status (inpatient versus outpatient), Karnofsky Performance Status (KPS), treated site and primary site.

Results: Of 973 patients, 389 (40%) received palliative RT; 30DM rate was 25.7% (n = 100). Median age was 61 and median KPS was 70. The most common primary sites were thorax (N = 121, 31.1%) and genitourinary tract (N = 60, 15.4%), while the most commonly treated sites were brain (N = 136, 35.0%) and bone (N = 71, 18.3%). For the palliative intent cohort (N=384), median duration of treatment was 11 days (0-50 days). For patients who had palliative intent treatment and died within 30 days of completing RT (N=97), median number of prescribed fractions was 9.34 compared to median number of prescribed fractions of 8.65 for palliative intent patients who did not die within 30 days of treatment (p=0.3). KPS and treatment site were strong independent predictors of both OS and 30DM. Patients with KPS < 70 had HR 2.61 (95% CI 1.54 – 4.43, p < 0.0001) for 30DM and HR of 2.22 (95% CI 1.57 – 3.13, p < 0.0001) for death at any time after RT. Treatment site was also independently associated with OS and 30DM (p = 0.01 and p = 0.03, respectively). Median OS durations were: abdomen/pelvis not reached, bone 340 days, spine 207 days, head and neck 184 days, brain 104 days and thorax 76 days. Age, sex, admission status and primary site were not correlated with OS or 30DM.

Conclusions: RT can effectively palliate distressing symptoms related to primary or metastatic cancers. However, early mortality is common following palliative RT and is associated with poor performance status and treated site. Hypofractionated RT courses should be considered in patients with such risk factors to reduce the burden of prolonged therapy on patients and their families at the end of life.

Behavioral ***Graduate Student***

POSTER #55

THE ASSOCIATION BETWEEN MATERNAL HISTORY OF CERVICAL CANCER AND HPV VACCINATION OF CHILDREN

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Background/Objectives: HPV vaccination uptake remains low, with only 54% of U.S. children receiving at least one dose of the vaccine by age 13. Prior research has demonstrated that parental health beliefs and sociodemographic factors predict a child's HPV vaccination status. Less is known, however, about the relationship between maternal history of cervical cancer and children's HPV vaccination. The purpose of this study was to explore (a) whether mothers' personal history of cervical cancer predicted HPV vaccine uptake in their children and (b) whether this association may be mediated (i.e., explained) by greater perceived benefits of HPV vaccination.

Methods: Data for this cross-sectional study were collected via an online survey conducted by Survey Sampling International. The survey targeted mothers or female guardians of children aged 9-13 living in the U.S. In a logistic regression model, we first estimated the effect of mothers' history of cervical cancer on maternal report of children's HPV vaccine uptake (i.e., at least 1 dose). Next, mothers' perceived benefits of HPV vaccination was included as a potential mediator of this relationship.

Results: Of the 1,155 women (aged 18-81) providing data about cancer history, 70 reported that they had ever received a cervical cancer diagnosis. Children were 59% female, 41% male, and 32% had received one or more doses of vaccine. Maternal history of cervical cancer predicted greater odds of child vaccination (OR=2.65, 95% CI=1.87-3.74). Higher levels of perceived benefits of HPV vaccination also predicted greater odds of child vaccination (OR=2.78, 95% CI=2.34-3.30). When controlling for perceived benefits of vaccination, the association between maternal history of cervical cancer and child's vaccination status remained significant (OR=2.86, 95% CI=1.68-4.87), suggesting that perceived benefits does not mediate the association between these two variables.

Conclusions: While mothers with a history of cervical cancer were more likely to vaccinate their children against HPV, this association does not appear to be due to greater perceived benefits of HPV vaccination. Mothers' HPV knowledge and/or perceived severity of cervical cancer (not measured in this study) could possibly contribute to this relationship. The current study builds on existing literature showing the link between HPV and cancer as a motivating factor in mothers' vaccination decisions. Clinicians and researchers may want to discuss HPV vaccination in the context of personal narratives about HPV-related cancers as a way of highlighting and humanizing the effects of non-vaccination.

Behavioral ***Graduate Student***

POSTER #56

THE ASSOCIATION BETWEEN SELF-REPORTED COGNITIVE ABILITIES AND COMMONLY REPORTED SYMPTOMS IN LONG-TERM BREAST CANCER SURVIVORS.

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Background: There are approximately 3.5 million breast cancer survivors (BCS) living in the US. These BCS can be left facing detrimental symptoms long into survivorship. We are interested in better understanding those commonly reported cancer symptoms.

Purpose: The purpose of this study is to examine the association between self-reported cognitive ability and commonly reported cancer symptoms in BCS. Specifically, we will examine the associations between multiple self-reported cognitive domains (language, visuo-perceptual, verbal memory, visual memory, and attention) and commonly reported cancer symptoms (anxiety, depressive symptoms, fatigue), while controlling for age and education.

Methods: This is a secondary data analysis of pooled data from 2 IRB-approved studies of BCS. 144 BCS met eligibility criteria and had complete data on the following questionnaires (symptoms): Multiple Ability Self-Report Questionnaire (cognitive abilities), Spielberger State Trait Anxiety Inventory - State (STAI-S) (anxiety), Centers for Epidemiologic Studies Depression Scale (CES-D) (depressive symptoms), and Functional Assessment of Cancer Therapy – Fatigue (fatigue). Data was analyzed using descriptive statistics, linear regression and change point models.

Results: BCS in this study were on average 54.4 (SD 8.8) years of age. The majority of the BCS were white (97.2%), married (66%), and working full-time (59.7%). In addition, these were long-term BCS, who were on average over 5 years post-treatment (5.1 years; SD 3.6) and well educated (some college - 15.6 years; SD 2.2). Anxiety was significantly related to cognitive abilities, with increased anxiety associated with poorer cognitive abilities ($p=0.0105-<0.0001$), except for visual memory, which, was noted to have an inflection point at which the slope changed and flattened at an anxiety score of 35. Depressive symptoms were significantly related to cognitive ability, with higher levels of depressive symptoms in general associated with poorer cognitive abilities ($p=0.045-<0.0001$). Regression models for depressive symptoms and cognitive ability showed a different slope on either side of depressive symptoms scores of 10 for visuo-perception, verbal memory, attention and total score. This indicating that values of 10 or less on the CES-D had a different slope than those scores equal to or below 10. Fatigue was significantly correlated with cognitive ability, with increased levels of fatigue associated with poorer cognitive abilities ($p<0.0001$). Age and time since treatment were not correlated with any of the cognitive ability outcomes. Highest education level showed statistically significant negative correlations with cognitive ability, but the correlation was weak ($p=0.0377-<0.0008$).

Conclusions: In general, increased levels of anxiety, depressive symptoms, and fatigue were associated with decrements in cognitive abilities. Potential cut-points on the STAI-S and CES-D were identified and if validated by future research could be used to screen BCS who may be more likely to have poorer cognitive abilities. Results from his study can inform future BCS survivorship care planning and future research.

Behavioral ***Graduate Student***

POSTER #57

SYMPTOM TREATMENT PREFERENCES OF CANCER SURVIVORS: DOES FATIGUE LEVEL MAKE A DIFFERENCE?

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Cancer-related fatigue (CRF) is one of the most common symptoms in cancer survivors and often co-occurs with other disabling symptoms, such as sleep disturbance, anxiety, and depression. Previous studies have examined various behavioral interventions for CRF, yet little is known about survivors' preferences regarding CRF treatment. To address this gap, we examined fatigued survivors' interest in learning skills to manage CRF and related symptoms as well as their interest in specific treatments in comparison to survivors with low fatigue.

Cancer survivors (N=343, 48% male) were recruited from academic medical centers and a public hospital in Indianapolis, IN. Survivors were mostly Caucasian (75%), with an average age of 62 years (SD=11). Diagnoses were early-stage (Stage I-II, n=172) or late-stage (Stage IV, n=171) breast, prostate, lung, or gastrointestinal cancer. Survivors completed measures of fatigue and treatment preferences. Based on standard cutoffs for fatigue severity and interference, survivors were classified as those with high (n=141) or low (n=202) fatigue.

A number of highly fatigued survivors reported interest in learning skills to manage fatigue (73%), fear of cancer recurrence (47%), and sleep problems (47%), as well as skills to manage depression, anxiety, stress, pain, and clouded thinking (26-36%). Regarding specific treatments, exercise and massage were the most common preferences for the high fatigue group (47-50%). A minority of highly fatigued survivors reported interest in mindfulness meditation, yoga, Tai Chi, resilient coping skills, self-compassion training, and acupuncture (24-33%). Chi-square tests were conducted to examine differences in treatment preferences between high and low fatigue groups. Compared to those with low fatigue, the high fatigue group was more likely to report interest in learning skills to manage fatigue, depression, anxiety, stress, sleep problems, pain, and clouded thinking (ps < .05). Those with high fatigue were also more likely to report interest in resilient coping skills training, self-compassion training, and acupuncture (ps < .05).

Findings suggest that many survivors, especially those with high fatigue, are interested in learning skills to manage CRF and related symptoms. Survivors' interest in exercise contrasts with the low uptake in exercise trials. Given the varied treatment preferences among survivors, findings provide support for testing a range of options for symptom management.

Behavioral ***Graduate Student***

POSTER #58

PROINFLAMMATORY CYTOKINES IN BREAST CANCER SURVIVORS AND CONTROLS: ASSOCIATIONS WITH PHYSICAL ACTIVITY AND COGNITION

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Physical activity may produce clinically meaningful improvement in cancer survivors' cognitive functioning. A growing body of research also shows that physical activity may reduce proinflammatory cytokines. Our aim was to determine if greater physical activity is associated with lower levels of circulating cytokines, and if lower cytokine levels are associated with fewer cognitive complaints and better cognitive performance in breast cancer survivors (BCS) and healthy controls (HC). Female non-metastatic BCS (n=24) and HC (n=23) completed neuropsychological assessments of attention and self-report surveys of weekly hours of moderate to strenuous physical activity and cognitive complaints, and gave blood samples for biomarker analysis. High sensitivity ELISA assays determined circulating levels of proinflammatory cytokines IL-1ß and IL-6; cytokine data were log transformed to reduce skewness. Two-sample *t* and ¿^{2}tests evaluated whether BCS differed from HC on demographics or any variable of interest, and separate regressions for BCS and HC tested hypotheses while controlling for age and Body Mass Index. BCS did not differ from HC on age (mean=59, SD=10), education, race/ethnicity, employment status, income, or any variable of interest with the exception of cognitive complaints. BCS reported significantly greater cognitive complaints than HC, $t(45)=-3.11$, $p<0.01$. In BCS, Metabolic Equivalent of Task (MET) hours of physical activity was negatively associated with IL-1ß ($\beta=-.59$, $p<.05$) and tended to be negatively associated with IL-6 ($\beta=-.38$, $p<.10$). In HC, physical activity was not significantly associated with IL-1ß or IL-6. In BCS, levels of IL-1ß were positively associated with cognitive complaints ($\beta=.75$, $p<.001$) and tended to be negatively associated with attention performance ($\beta=-.39$, $p<.10$), and levels of IL-6 were not associated with cognitive complaints or attention performance. No significant associations were found in HC. In conclusion, proinflammatory cytokines appear to be related to physical activity and cognition in BCS, but not HC. Future research may determine if different findings for BCS and HC could be explained by the effect of cancer and its treatment on cognitive impairment and cytokine deregulation. Additionally, future research should use larger samples to determine whether decrease in proinflammatory cytokines is a potential biological mechanism for the role of greater physical activity in improving cognitive function for BCS.

Behavioral ***Graduate Student***

POSTER #59

INCREASING MINORITY ONCOLOGY REPRESENTATION (MORE) IN CLINICAL TRIALS

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Clinical trials are scientifically significant for the safe development and evaluation of new treatments for debilitating diseases such as cancer. For this reason, minority representation is essential to decrease ethnic and racial disparities in cancer outcomes (Chen et al, 2014). The National Institute of Health (NIH) Revitalization Act of 1993 was implemented to combat issues caused by recruitment barriers by enforcing that women and minorities are proportionately included in all NIH-funded clinical research studies. To date, minorities remain underrepresented while having disproportionately higher rates of chronic diseases (Heller et al, 2014). Clinicaltrials.gov enrollment data showed a decrease in minority accruals, specifically African Americans, between 2003 and 2016 (Duma et al, 2018). As minority populations continue to increase in the United States, their representation in clinical trials is becoming imperative to decrease disproportionate cancer burdens within minority groups (Chen et al, 2014).

Low participation and representation in clinical trials among minority populations, indicated in local and national clinical trial accrual data, is caused by provider, system and patient barriers but mediated by awareness and knowledge given that appropriate educational programs set in place for providers and patients moderate the causes. The socioeconomic factors, genetic pre-disposition, lack of access or knowledge to clinical trials, and historical mistrust in providers, exist prior to the causes.

Goal 1: Provide awareness of clinical trials for academic fellows by engaging fellows

Goal 2: Increase the number of minorities recruited and enrolled onto clinical trials at IUSCC and Eskenazi Health

Objective 1. By the end of Q2, all current fellows and faculty will be aware of current and upcoming clinical trials available at IUSCC and Eskenazi Health through the use of a clinical trial database

Objective 2. By the end of Q3, minority accruals onto hematology/oncology clinical trials will have increased by an overall 5% at the IUSCC and Eskenazi Health recruitment sites

Increased collaboration and communication will occur between clinical disease oriented teams (DOT), academic fellows and other clinical trial staff at IUSCC and Eskenazi Health over the next 18 months starting in January 2019. Use of Epic software, creation of a clinical trial database, staff attendance and participation in monthly DOT meetings as well as a review of trial portfolios will solidify outcomes.

A pre and post evaluation survey will be conducted using Redcap and distributed to current fellows March and July of 2019 to assess for changes in attitudes, behaviors and awareness of clinical trials available at IUSCC and Eskenazi Health. As new fellows rotate through their academic training, a baseline evaluation will be conducted on month 1 and comparison at the end of the month 6 to look for changes in provider attitudes and awareness as well as accrual increases in minority populations.

Behavioral ***IUSCC Staff/Graduate Student***

POSTER #60

PARENTS OF HEALTHY CHILDREN ASSIGN LOWER QUALITY OF LIFE MEASURE TO SCENARIOS LABELED AS CANCER THAN TO IDENTICAL SCENARIOS NOT LABELED AS CANCER

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Background: While it is commonly understood that a cancer diagnosis evokes feelings of fear, the effect of labeling a child's illness as "cancer" remains unstudied. We hypothesized that lower health utility scores would be assigned to disease states labeled as cancer compared to identical disease states without the mention of cancer.

Methods: In this randomized study, caregivers of healthy children were asked to assign health utility values to different scenarios written as improving, stable, or worsening. Participants from general pediatric clinics at Eskenazi Health were randomly assigned to either the scenarios labeled as "cancer" or "a serious illness". Participants then rated the scenarios using the Standard Gamble, with laddering of health utilities between 0 (a painless death) and 1 (perfect health). We also gathered subject demographics and assessed the subject's numeracy.

Results: We approached 319 subjects and 167 completed the study. Overall median health utilities of "cancer" scenarios were lower than "serious illness" scenarios (0.61 vs. 0.72, p=0.018). Multivariate regression (with an outcome of having a utility above the 75thpercentile) showed no significant effects by race, ethnicity, numeracy, or income level. "Cancer" scenarios remained significantly lower after adjustment for confounders using logistic regression, but only for the more serious scenarios (OR 0.92, p=0.048).

Conclusions: On average, caregivers with healthy children were shown to take more risk with their treatment options and view their child as having a worse quality of life when they knew the disease was cancer. Awareness of this bias is important when discussing treatments with families, particularly when a risk of cancer is present.

Behavioral ***Medical Student***

POSTER #61

THE EXAMINATION HPV VACCINE LITERACY AND ADMINISTRATION RATES IN THE IMMEDIATE POSTPARTUM PERIOD

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Background: Despite a decrease in the number of human papilloma virus (HPV)-associated cancers since the introduction of the HPV vaccine in 2006, vaccination rates have remained well below the Healthy People 2020 goal of 80%. Barriers to HPV vaccination are evident and range from financial concerns to preconceived notions of the vaccine. In Indiana, HPV vaccine rates are below the national average. In 2017, only 41% of adolescents were up to date on their HPV vaccine. Data is varied on whether HPV literacy increases vaccination administration rates.

Hypothesis: Initiation of HPV vaccination series in the postpartum period will increase the vaccination rate at Eskenazi Health Clinics. Women who have higher HPV literacy rates will have higher vaccination and intent to vaccinate rates.

Methods: Expectant mothers aged 18-45 will be counseled by a physician on HPV vaccination at their 28 week prenatal visit at Eskenazi Health OBGYN clinics. Additionally, an informational pamphlet will be distributed in their native language (English, Spanish). Following delivery during postpartum hospitalization, the EPIC EMR will prompt the administration of the first dose of the HPV vaccine administration with plans to complete the series at her routine postpartum visit and a scheduled nurse visit.

A REDCap survey will be administered during postpartum hospitalization to examine HPV literacy as well as intent to vaccinate for all women aged 18-45. HPV literacy status will be compared with reported intent to vaccinate, HPV vaccine uptake, and various data points including delivery information, socioeconomic and insurance status, pap smear history, other vaccination status to examine possible barriers to vaccine uptake.

Conclusions: This physician and patient centered intervention is designed to increase adherence to standard of care recommendations surrounding the HPV vaccination series and assess areas of possible intervention. We anticipate a clinically significant increase in vaccination rates at Eskenazi health OBGYN clinics with women who demonstrate higher HPV literacy rates having higher vaccination and intent to vaccinate rates. Results anticipated in Fall 2019.

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

POSTER #62

THE IMPACT OF ORAL ONCOLYTIC AGENT DOSING ON CANCER PATIENT SYMPTOM PROFILES

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Purpose: Oral Oncolytic Agents (OOA) are frequently prescribed as third of fourth line therapy, often replacing or in combination with intravenous (IV) chemotherapy. Because of disease status or symptom profile, dosage strengths may not follow FDA recommendations resulting in increased symptom burden. OOAs are sometimes prescribed at higher than FDA-recommended doses due to pill size availability. For these reasons, there is a need to examine the impact of increased doses on symptom burden.

Questions/Hypothesis: To examine the impact of the proportion of initially prescribed OOA dose to FDA-recommended dose on symptom severity in cancer patients newly-prescribed OOAs. It is hypothesized that patients prescribed higher proportions of initial OOA dose will experience a greater symptom burden than those prescribed lower proportions.

Methods: A total of 272 cancer patients newly prescribed OOAs (136 males, 136 females) were entered into a multi-site RCT testing adherence and symptom-management interventions after signing written consent. Survey data including medication information and symptom severity (range: 1-10) of 18 symptoms using Symptom Experience Scale were collected via telephone interviews at baseline and four weeks post-intervention delivery. Patients reporting symptom severity =4/10 were provided the Symptom Management Toolkit. A multiple linear regression model was used to test the effect of age, sex, comorbidities, trial group, OOA drug class, baseline symptom severity, and proportion of initial prescribed OOA dose to FDA-recommended dose on symptom severity at 4 weeks.

Results: Patients had a mean age of 61 years and an average of 3.38 comorbidities, in addition to a cancer diagnosis. The mean proportion of initial OOA prescribed dose to FDA-recommendation was 0.91 (range: 0-4.12). Mean symptom severity at baseline was 23.61 (0-117), while the mean at 4 weeks was 22.19 (0-122). Only baseline symptom severity ($P<.01$) and initial dose proportion ($P<.01$) significantly predicted symptom severity at 4 weeks.

Conclusions: Data analysis confirmed that individuals prescribed a higher initial dose proportion experienced a greater symptom burden than those prescribed a lower initial dose. Prescribers should take into account baseline symptom burden when prescribing initial OOA doses; higher prescribed doses lead to higher symptom burden, which may result in regimen modifications as treatment continues. Oncology nurses should recognize patients' symptom level at initiation of OOA in order to promote proactive symptom management and reduce poor outcomes over time.

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

POSTER #63

CUTANEOUS NEVI AND CANCER RISK: RESULTS FROM TWO LARGE PROSPECTIVE COHORTS OF US WOMEN

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Importance: Longer telomeres have been linked to elevated cutaneous nevus number. Recently, a large Mendelian randomization study identified a positive association between telomere length and risk of cancer. We hypothesized that higher nevus count, as a phenotypic marker of longer telomere length, may be associated with increased risk of cancer.

Objective: To investigate prospectively the association of extremity nevus count with risk of total and site-specific cancer.

Design, Setting, and Participants: The Nurses' Health Study (NHS) and Nurses' Health Study II (NHS2) are two prospective ongoing cohorts of US female nurses. In the present analyses, the baselines in the NHS and NHS2 were 1986 and 1989, respectively, when number of nevi was measured. A total of 74,507 women from NHS and 104,844 women from NHS2 were followed for more than 30 years. During 3,941,477 person-years of follow-up, we documented a total of 24,428 cancer cases (16,297 in the NHS and 8,131 in the NHS2). Cox proportional hazards regression model was used to estimate the age-adjusted and multivariate-adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) for cancer risk associated with nevus count.

Exposures: Nevus count on extremities.

Main Outcomes and Measures: Hazard ratios for incident cancer.

Results: Compared to participants who had no nevi, the multivariate HRs of total cancer were 1.08 (95% CI, 1.05-1.11) for women with 1-5 nevi, 1.15 (95% CI, 1.09-1.21) for those who had 6-14 nevi, and 1.26 (95%, 1.18-1.34) for those with 15 or more nevi (p trend < 0.0001). Moreover, because nevus count has been associated with risk of melanoma and breast cancer in previous epidemiological studies, we conducted secondary analyses by excluding 1) only cutaneous melanoma and 2) both melanoma and breast cancer from the outcomes of interest, respectively. The results of these analyses were very similar to those of our primary analysis using all cancer sites. For individual cancer sites, most of the associations with mole counts were positive but not statistically significant.

Conclusion and Relevance: We identified the number of cutaneous nevi as a phenotypic marker associated with cancer risk, which may possibly be explained by telomere biology.

Population Science/Epidemiology ***Faculty***

POSTER #64

HEIGHT, NEVUS COUNT, AND RISK OF CUTANEOUS MALIGNANT MELANOMA AMONG US WOMEN

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Abstract

Background: Previous observational studies have reported an elevated risk of melanoma among taller individuals. However, evidence on the association between height and nevus count, a well-known phenotypic marker of melanoma risk, has been very sparse. Moreover, no study has quantified the degree to which nevus count explains the excess melanoma risk associated with increased height.

Methods: We used Cox proportional hazards models to prospectively examine the risk of melanoma in relation to adult height in the Nurses' Health Study (NHS) and the Nurses' Health Study 2 (NHS2). We also investigated the association between height and nevus count using multinomial logistic regression models. Proportion of association explained by nevus count was calculated by comparing hazard ratios for the height-melanoma association, with and without adjustment for nevus count.

Results: Results for association between height and melanoma risk were not significantly different between the two cohorts. In fixed-effect meta-analysis, the hazard ratio (HR) was 1.22 [95% confidence interval (CI): 1.12, 1.33] for the association between every 10-cm increase in height and risk of melanoma, after adjustment for potential confounders. Height was significantly positively associated with nevus count in both cohorts, although significant heterogeneity was detected. Compared to women with no nevi, the multivariable-adjusted odds ratios (ORs) associated with 10-cm increase in height were 1.35 (95% CI: 1.23, 1.49) in the NHS and 1.12 (1.00, 1.06) in the NHS2 for women with 10+ moles. The estimated proportion of excess melanoma risk associated with a 10-cm increase in height explained by nevus count was 7.9% in the NHS and 9.2% in the NHS2, adjusting for other potential confounders (P-value for mediation effect < 0.0001).

Conclusion: Nevus count is an important explanatory factor for the excess risk of melanoma among taller people. Future studies on early-life exposures and genetic markers of pleiotropic effects may provide more biological insights into the connections among height, nevus count, and melanoma.

Population Science/Epidemiology ***Faculty***

POSTER #65

GENOME-WIDE META-ANALYSIS IDENTIFIES EIGHT NEW SUSCEPTIBILITY LOCI FOR CUTANEOUS SQUAMOUS CELL CARCINOMA

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Cutaneous squamous cell carcinoma (SCC) is one of the most common cancers with an estimated 700,000 cases diagnosed in the United States annually. Metastatic SCC is responsible for 3900-8800 deaths annually in the United States. Risk factors for SCC include age, gender, pigmentation phenotypes, ultra-violet radiation exposure, and immunosuppression. Recently, three genome-wide association studies (GWAS) have identified 14 single nucleotide polymorphisms (SNPs) associated with cutaneous SCC. We conducted the largest cutaneous SCC meta-analysis to date, representing 6 international cohorts and totaling 19,149 SCC cases and 680,049 controls. In addition to confirming 14 previously reported loci, we discover 8 novel loci associated with cutaneous SCC. In total the twenty-two loci explain 8.5% of heritable risk for SCC. Subanalyses of these 22 loci identify 9 loci associated with pigmentation phenotypic traits and 1 locus (*HAL*) associated with photodistribution-specific risk. In addition, fine mapping identifies potentially causal SNPs which fall within putative regulatory elements in keratinocytes and melanocytes and regulate the expression of genes involved in cancer progression, differentiation, and immune regulation, highlighting the role of these pathways in modulating SCC susceptibility.

Population Science/Epidemiology ***Faculty***

POSTER #66

SYMPTOM CLUSTERS AND SURVIVAL IN BREAST AND COLORECTAL CANCER SURVIVORS: USING A LARGE INDIANA CLINICAL DATABASE.

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Introduction: Breast cancer (BC) and colorectal cancer (CRC) are two of the four most common cancers in Indiana. BC and CRC patients experience symptoms that affect their quality of life and overall survival. Yet, large available clinical databases have not been utilized to fully examine symptom clusters and survival rates. The purpose of this study is to describe the symptom clusters and survival rates of BC and CRC patients using a large health system database.

Study Cohort: The IU Health based electronic medical record (EMR) database was linked to Indiana State Cancer Registry to identify BC (stage I-III) and CRC (stage II-III) patients diagnosed at age 21 years and older in 2007 – 2017. Clinical notes were identified based on keyword search for terms related to fatigue, pain and depression. In total, there were 58,419 clinical notes for 3,430 BC patients and 26,278 clinical notes for 1,617 CRC patients.

Methodology: Time-to-event analyses were performed using Kaplan-Meier method for the overall survival of BC and CRC patients from the date of diagnosis. To explore the reported symptom profile, we built a two-level clustering framework to identify the clusters based on the occurrences of symptoms within a timeframe. UMLS MetaMap was used to extract the concepts in semantic categories from the raw clinical notes. Deep learning-based term embeddings were used to convert the symptoms into vectors. The k-means clustering algorithms were used to generate symptom clusters that were grouped by body location. The tf-idf values based on the symptom clusters and normalized demographic, vital and comorbidity features were calculated for patient presentation.

Results: Two cohorts of 4,565 BC and 2,163 CRC patients were identified. We successfully identified symptom clusters for fatigue, pain and depression. There are more clusters for pain and peripheral neuropathy type. The visualization of the clusters demonstrates some overlap between the groups, which shows the co-occurrences of the symptoms in the patient profiles. The patient clustering results show different patient profiles with different symptoms and demographic, vital and comorbidity features after chemotherapy. The estimated 10-year survival rates from the date of diagnosis for BC and CRC are 80% (95%CI: 78% - 82%) and 61% (95%CI: 56% - 65%), respectively. The estimated 5-year survival rates for BC and CRC are 87% (95%CI: 85% - 88%) and 78% (95%CI: 75%-80%), respectively.

Conclusion: Symptom clustering generated different profiles for BC and CRC patients. Analysis of large datasets can be informative in identifying symptoms by cancer diagnosis. The estimated 5 and 10-year survival rates for BC are a little lower than the average national 5 and 10-year survival rate, 90% and 83% respectively. However, the estimated 5-year survival rate for CRC is much higher than the average national 5-year survival rate, 65%.

Population Science/Epidemiology ***Faculty***

POSTER #68

ASSESSING THE EFFECTIVENESS OF ALTERNATIVE TREATMENT OPTIONS FOR BREAST CANCER BASED ON CLINICAL STAGES: A QUALITATIVE METHODOLOGICAL APPROACH

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ABSTRACT

Introduction: Breast cancer is one of the most commonly diagnosed cancers in American women. Recent estimated trends show that around 268,600 new cases of invasive breast cancer are expected to be diagnosed, and around 42,260 breast cancer deaths will occur in the US in 2019. To promote effective choice of treatments among patients, we study the time ratio and the hazard ratio between the survival of surgical methods, Lumpectomy and Mastectomy, after adjusting for various characteristics including tumor behavior, lymph nodes containing metastases, tumor size, radiation status, hormone-receptor status, and cancer stage.

Methods: This study uses the 1990-1997 Surveillance, Epidemiology and End Results (SEER) incidence dataset including 21,847 insured respondents, mostly white women of age 19-105 years, diagnosed with stage 0-IV breast cancer and have undergone either Lumpectomy or Mastectomy. We use Kaplan Meier Survival Curve to estimate the unadjusted probability of survival over time, Exponential and Log-logistic models to adjust for the full set of covariates and calculate time ratios which is a comparison of rates at which subjects traverse the survival curve. Finally, we use Cox Proportional Hazard (PH) model to investigate the rate of survival time of patients opting for any of the two treatments, given the stage of breast cancer and the predictor variables. PH model fit was assessed using the log-log survival, the score process, and the supremum test. We also conduct likelihood displacement to examine the existence of any influential outlier.

Results: Kaplan Meier estimation shows that there is no significant difference in the survival rate between the two treatments in earlier stages (0 and I) of breast cancer. In stages II and III, patients mostly opt for Mastectomy although patients undergoing Lumpectomy has statistically higher survival rates. In stage IV, patients choosing Mastectomy has higher survival rate although they tend to choose Lumpectomy more. Compared to Mastectomy group, patients undergoing Lumpectomy will survive 1.2 times longer. Compared to patients who were in stage IV, those in early stages will survive 4.7 to 15.5 times longer, depending on the severity of cancer. Patients with larger localized tumor has a 1.1 to 1.3 times longer survival time compared to those with smaller yet spread tumor. Patients with non-localized cancer, opting for radiation before surgery has 1.2 times longer survival time compared to patients with localized cancer not opting for radiation. After considering the modifier effect, Mastectomy has higher hazard (stage 0, 1.45; stage I, 1.55; stage II, 1.70; stage III, 1.44) except for patients at stage IV (0.83). We find that the PH assumption for treatment has not been violated.

Conclusion: Adjusting for the risk factors and the modifiers, we find that patients choose Mastectomy over Lumpectomy in stages II and III even when the latter is more effective and those in stage IV opt for Lumpectomy even though Mastectomy is more effective. These results agree with the literature that shows the reasons to such decisions as follows: (i) patients in earlier stages prefer Mastectomy mostly out of fear of recurrence and the financial burden of going through Lumpectomy followed by five to six weeks of radiation; (ii) patients in stage IV care for their quality of life and rather select the less invasive treatment.

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

POSTER #69

ASSOCIATION BETWEEN CALCIUM INTAKE AND PANCREATIC CANCER RISK IN THE PROSTATE, LUNG, COLORECTAL, AND OVARIAN CANCER SCREENING TRIAL (PLCO)

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Pancreatic cancer is a leading cause of cancer death in the U.S. and its etiology remains largely unclear. As most patients are diagnosed with an advanced, unresectable disease, primary prevention remains a priority for reducing its incidence and mortality. Although ecological, migrant, and temporal trend studies have shown that diet plays a role in the occurrence of pancreatic cancer, few nutrients that alter its risk have been identified from epidemiologic studies. The present study thus sought to investigate intakes of calcium and fat, adiposity, and their potential interactions in relation to pancreatic cancer risk among participants in the PLCO trial. Usual dietary intake among the participants was assessed with Dietary Questionnaire (DQX) at baseline and Dietary History Questionnaire (DHQ) around their third anniversary of randomization. Of 58,477 participants who completed the DQX, 279 cases of pancreatic cancer were documented during a median follow-up of 12.2 years. A total of 101,721 participants responded to the DHQ and gave risk to 380 cases of pancreatic cancer during a median follow-up of 8.9 years. Cox proportional hazards regression was performed to estimate hazard ratios (HR) and 95% Confidence intervals (CI) for pancreatic cancer in relation to total and dietary intake of calcium, intake of fat, and body mass index (BMI). After adjustment for established and suspected confounders, there was a suggestive inverse association between total calcium intake assessed from both food frequency questionnaires and risk of pancreatic cancer [HR (95% CI) for quartile (Q) 2, Q3, and Q 4 vs. Q1: 0.93 (0.67, 1.30), 0.86 (0.60, 1.24), and 0.71 (0.46,1.09); p-trend, 0.11) for DQX and 0.96 (0.72, 1.26), 0.99 (0.74, 1.33), and 0.72 (0.52, 1.01); p-trend, 0.08) for DHQ]. Overall, this inverse association was stronger or its linear trend in reduced risk across the quartiles of total calcium intake was more apparent among overweight participants (BMI: >25-<30) [e.g. HR (95% CI) for Q2, Q3, and Q4 vs. Q1: 0.91 (0.60, 1.38), 0.84 (0.54, 1.30), and 0.72 (0.44, 1.18); p-trend, 0.07 for DHQ]. Among the participants with the highest fat intake (Q4) derived from the DHQ, those with the highest intake of total calcium (Q4) experienced a 65% reduced risk of pancreatic cancer compared with those with the lowest intake (Q1) [HR (95% CI): 0.35 (0.17, 0.68)]. However, the possibility of chance finding for this significant risk reduction could not be ruled out due to multiple comparisons. In addition, no appreciable associations of calcium intake from both dietary and supplemental sources with pancreatic cancer risk was observed. In summary, the present study offers suggestive evidence that total calcium intake was associated with a reduced risk of pancreatic cancer and that this potential benefit may be more pronounced among overweight subjects.

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

POSTER #70

SMOKING AND MAMMOGRAPHY SCREENING: THE ROLES OF KNOWLEDGE AND HEALTH BELIEFS

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Introduction: Breast cancer is the second leading cause of cancer death among women in the United States. Although mammography screening is supported by the U.S. Preventive Services Task Force and has been found to effectively reduce mortality, only about 65% of women over 40 years of age are adherent to mammography screening guidelines, a rate far below the 81.1% goal proposed for Healthy People 2020. Screening rates are even lower among smokers compared to non-smokers; however, the reasons for this disparity remain unclear. Overall, knowledge, barriers, benefits, self-efficacy, and perceived risk play a role in decision making and may influence cancer screening behavior. Therefore, the purpose of this study was to examine the association between smoking status and mammography adherence, and to determine whether differences in knowledge, barriers, benefits, self-efficacy, and perceived risk could account for screening disparities between smokers and non-smokers.

Methods: A prospective, randomized screening intervention of women aged 50-75 (n=1,196) was conducted in Indiana from 2013-2015. A total of 846 women had complete smoking and mammography data for use in this analysis. Women were surveyed at baseline to ascertain current smoking status and mammography history, as well as knowledge, barriers, self-efficacy, benefits, and perceived risks for mammography. Binary logistic regression was used to estimate multivariate-adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between smoking and mammography adherence. T-tests were used to evaluate differences between current smokers and non-smokers with regard to knowledge, barriers, benefits, self-efficacy, and perceived risk. Regression analyses were adjusted for age, education, income, insurance coverage, ever receiving a physician recommendation for mammography, and family history of breast cancer.

Results: Smokers were significantly younger, less educated, and had lower income relative to non-smokers. Smokers were less likely to be adherent to mammography screening relative to non-smokers [OR = 0.54 (0.32 – 0.90), p=0.020)]. Smokers also demonstrated significantly less knowledge of breast cancer and mammography (p=0.0016), reported more barriers to screening (p=0.020), had less self-efficacy (p=0.0018), and had a lower perceived risk of breast cancer (p=<0.0001) compared with non-smokers. No differences were observed for benefits to screening (p=0.81).

Conclusions: Women who smoke are less likely to adhere to mammography screening guidelines than non-smokers, possibly due to less knowledge about breast cancer and mammography, more barriers to screening, less self-efficacy, and less perceived risk. Future interventions to increase mammography should consider smoking status disparities and tailor intervention content accordingly.

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

POSTER #71

PRE-DIAGNOSTIC LEUKOCYTE MITOCHONDRIAL DNA COPY NUMBER AND COLORECTAL CANCER RISK

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Mitochondrial DNA (mtDNA) is particularly vulnerable to oxidative stress and mutations. As a promising biomarker of oxidative stress-related health outcomes, mtDNA copy number (mtDNAcn) in peripheral blood leukocytes has been associated with a range of diseases, including cancers. Few epidemiological studies have assessed the relationship between mtDNAcn and risk of colorectal cancer (CRC), with inconsistent findings, while evidence in western populations has been lacking. We examined the association between pre-diagnostic leukocyte mtDNAcn and CRC risk in a case-control study of 324 female cases and 658 matched controls nested within the Nurses' Health Study (NHS). Relative mtDNAcn in peripheral blood leukocytes was measured by quantitative PCR-based assay. Conditional logistical regression models were applied to estimate adjusted odds ratios (AORs) and 95% confidence intervals (95% CIs) for the association between log-transformed mtDNAcn (log-mtDNAcn) and CRC risk. We found that lower log-mtDNAcn level was significantly associated with an increased risk of CRC, with a dose-dependent relationship (*P* for trend < 0.0001). Compared to the highest (4^{th}) quartile, AOR (95% CI) was 1.09 (0.68, 1.75) for the 3^{rd} quartile, 1.38 (0.88, 2.17) for the 2^{nd}quartile, and 2.17 (1.42, 3.33) for the 1^{st} quartile. In the further analysis by anatomic subsite of CRC, we found a significant inverse association for proximal colon cancer [lowest vs. highest quartile, AOR (95% CI) =3.26 (1.67, 6.38), *P* for trend = 0.0004]. Moreover, we observed an effect modification of Alternate Health Eating Index (AHEI) on the association between mtDNAcn and CRC risk (*P* for interaction = 0.03); the significant inverse association of mtDNAcn and CRC was only detected among individuals in the lowest AHEI tertile group [lowest vs. highest mtDNAcn quartile, AOR (95% CI) = 3.74 (1.74, 8.03), *P* for trend = 0.001] but not among those in the 2^{nd} and 3^{rd} AHEI tertile groups. No significant effect modification appeared for other potential confounders on the association between mtDNAcn and risk of CRC. Additionally, we performed a stratified analysis according to the follow-up time since blood collection (i.e., mtDNAcn testing). The inverse association between mtDNAcn and CRC remained significant among individuals with = 5 years' follow-up since mtDNAcn testing [lowest vs. highest quartile, AOR (95% CI)= 1.98 (1.18, 3.33), *P* for trend = 0.009]. The inverse association was also marginally significant among those with = 10 years' follow-up [lowest vs. highest quartile, AOR (95% CI)= 1.94 (0.94, 4.00), *P* for trend = 0.06], suggesting that mtDNAcn may serve as a long-term predictor for the risk of CRC. In conclusion, pre-diagnostic leukocyte mtDNAcn was inversely associated with CRC risk. Further basic scientific research is needed to explore the underlying biological mechanisms of mtDNAcn on CRC carcinogenesis.

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

POSTER #72

MISSED FOLLOW UP IS AN INDEPENDENT PREDICTOR OF SURVIVAL IN STAGE I LUNG CANCER: RESULTS FROM A LARGE MULTI-SITE ACADEMIC INSTITUTIONAL STUDY

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Purpose/Objectives: Lung cancer is the leading cause of cancer mortality in the United States. Regular oncologic follow-up is recommended to monitor patients for late side effects of treatment, resolution of symptoms, and recurrence, especially since salvage therapy may be more effective if recurrences are detected early. Previous work has identified patient and disease characteristics associated with missed follow up. This study aimed to examine the effect of incomplete follow up on survival in patients with early stage lung cancer.

Materials/Methods: Appointment data for patients with solid tumors diagnosed in our statewide health system was linked with cancer registry data with institutional IRB approval. This analysis included patients diagnosed with stage I lung cancer between 2007 and 2016 and excluded patients who had missing clinical, personal or appointment data, were <18 years old, or were treated for multiple cancers. Patients were classified as having a missed follow up if they had a no show, canceled or rescheduled appointment without a subsequent attended appointment within 60 days. Missed appointments were excluded if they were within 30 days of patient death. Survival analysis was performed using a Cox regression model to investigate the effect of missing >10% of appointments (80^{th} percentile) on survival while adjusting for age, sex, race, treatment modality, Charlson Comorbidity Score, and insurance status.

Results: 783 patients were analyzed. Median age was 70 years; 9.5% of patients were African American, 43.4% were male, 76.5% had a Charlson score of zero; 49.7% received surgery, 26.2% received SBRT, 15.3% fractionated RT and 8.8% received no treatment. Overall, 18.3% of patients missed >10% of follow up appointments. On multivariable survival analysis, patients missing >10% of follow up appointments had an increased risk of death (HR 1.39 [95% CI 1.08-1.78; p=0.01]). Age was associated with increased risk of death, with HR 1.24 [95% CI 1.09-1.41; p=0.001] for every 10 years increase, while female sex was associated with decreased mortality (HR 0.67 [95% CI 0.54-0.83; p<0.001]). Treatment with fractionated RT (HR 3.31 [95% CI 2.46-4.47; p<0.001]), SBRT (HR 2.62 [95% CI 1.98-3.49; p<0.001]), and no treatment (HR 4.11 [95% CI 2.83-5.98; p<0.001]) were associated with worse survival compared to patients who underwent surgery. Race, Charlson score, and insurance status did not significantly affect survival in our model.

Conclusions: To our knowledge, this is the first study to correlate oncology follow up appointment attendance with survival in any cancer site. Patients with stage I lung cancer who missed >10% of oncology follow up appointments had significantly worse survival than patients who attended a high proportion of their appointments. These results combined with previous work identifying at risk groups for missing follow up should motivate research into improving follow up for patients at risk of missing appointments.

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

POSTER #73

DISTANCE FROM CANCER FACILITY AS A BARRIER TO TIMELY TREATMENT AMONG PATIENTS WITH NON-METASTATIC CERVICAL CANCER

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Purpose/Objectives: In the United States cervical cancer cases have diminished due to vaccination and screening, however it remains the second most common cause of cancer death among women age 20-39 years. Distance from treatment facility could contribute to treatment delay and ultimately disease outcomes. In this study we hypothesize that greater distance from treatment facility results in a delay in treatment among patients with non-metastatic cervical cancer.

Materials/Methods: Data from the National Cancer Database were used which includes patient demographics, disease characteristics, and first course treatment decisions. Patients with non-metastatic cervical cancer between 2004 and 2015 treated with definitive radiation or surgery were included. Patients were excluded if they had missing demographic or disease information, variant histology, treatment >180 days from diagnosis, or lived >1000 miles from the treatment facility resulting in 36,986 patients. Distance was categorized by quartiles. Univariate comparisons were performed using chi-square and analysis of variance. A multivariable linear regression model was used to investigate the effect of distance on time from diagnosis to initiation of treatment while adjusting for age, insurance status, race and ethnicity, income quartile, education, urbanization, Charlson Comorbidity Score, year of diagnosis, stage, and initial treatment modality.

Results: The mean age was 49.5 years, 16.2% of patients were Black, 14.2% were Hispanic, 48.7% of patients had private insurance, 98.4% of patients lived in urban or metro counties, and 56.1% of patients received surgery as opposed to 43.9% who received radiation as initial treatment. Multivariable analysis identified a delay in treatment of 1.1 days for distance quartile 2 ($p=0.008$), 2.0 days for quartile 3 ($p<0.001$), and 4.0 days for quartile 4 ($p<0.001$) compared to patients in the closest quartile. Black patients (2.3 days, $p<0.001$) and Hispanic patients (5.1 days, $p<0.001$) experienced significant delays compared to white non-Hispanics. Patients with Medicaid (4.9 days; $p<0.001$) and who were uninsured (4.7 days; $p<0.001$) were both treated later than patients with private insurance. Patients living in rural counties were treated over 8.5 days earlier than those from the most populous metropolitan counties ($p<0.001$).

Conclusions: Greater distance from treatment facility resulted in a statistically significant delay in treatment. Because treatment delay has been associated with worse outcomes, these results could motivate clinicians to identify patients living further from their facility as being higher risk for a delay in treatment. Further research is needed to fully characterize the effect of distance from facility on ultimate disease outcomes in cervical cancer.

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

POSTER #74

DRUG USE AND RISK OF ALL-CAUSE MORTALITY AMONG PATIENTS WITH MALIGNANT MELANOMA:DATA FROM ELECTRONIC MEDICAL RECORDS

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Background: Malignant melanoma (MM) is the least common skin cancer but with the greatest mortality rates. These patients always have other comorbid diseases (e.g., hypertension and diabetes) and take numbers of drugs. This study aimed to evaluate the association between commonly used drugs and risk of all-cause mortality among the patients with MM using the data from electronic medical records.
Methods: We selected Tumor Registry confirmed MM cases diagnosed between 1960 and 2017 from the Vanderbilt University Medical Center and extracted their drug information from electronic medical records. We evaluated the following commonly used drugs: stains, nonsteroidal anti-inflammatory drug (NSAIDs), proton-pump inhibitors (PPIs), glucose-lowering drugs (excluding insulin), insulin, and antihypertensive drugs (including angiotensin-converting-enzyme inhibitor (ACEIs), angiotensin II receptor blockers (ARBs), beta-blockers, calcium channel blockers (CCBs), and diuretics). We used logistic regression to compute odds ratios (ORs) for all-cause mortality with 95% confidence intervals (CIs) comparing ever users with non-users.
Results: In total, 3,721 patients with MM were included in this study, of whom 698 died. Risk of all-cause mortality decreased with statin use (OR = 0.44, 95%CI = 0.36 - 0.54, $p < 0.0001$), NSAID use (OR = 0.76, 95%CI = 0.61 – 0.93, $p = 0.008$), ACEI use (OR = 0.74, 95%CI = 0.60 – 0.90, $p = 0.0035$), and ARB use (OR = 0.64, 95%CI 0.50 – 0.84, $p = 0.0009$) after adjusting for age at diagnosis of MM, sex, primary site, histologic type, race, and other comorbid diseases including other cancer, cardiovascular disease, hypertension, diabetes. However, diuretic use (OR = 1.43, 95%CI = 1.18 - 1.73, $p = 0.0003$), PPI use (OR = 1.20, 95%CI = 1.00 – 1.44, $p = 0.049$), and insulin use (OR = 2.48, 95%CI = 1.99-3.09, $p < 0.0001$) were significantly associated with increased risk of all-cause mortality.
Conclusion: This study found a decreased risk of all-cause mortality associated with statin use, NSAID use, or ARB use while an increased risk among those taking diuretics, PPIs, or insulins. However, given several limitations in this study, further prospective studies are required to confirm these findings.

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

POSTER #75

VALIDATION OF THE MYC-RAD21 COPY NUMBER VARIATION (CNV) AS A THERAPEUTIC BIOMARKER IN RECURRENT PEDIATRIC OSTEOSARCOMA.

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Osteosarcoma (OS), the most common pediatric bone cancer, has a 5-year survival rate of < 30% due in part to therapeutic resistance mechanisms that stem from copy number variations (CNVs). Our large-scale query of publicly available datasets indicate that amplifications of MYC and RAD21 genes on chromosome 8q correlated with poor overall survival in pediatric OS patients.

MYC is a well-known oncogenic transcription factor in OS that can be indirectly targeted by bromodomain inhibitors (BETi). RAD21, a chromatid cohesion complex protein, is associated with DNA replication, transcription, and repair pathways. However, no RAD21 inhibitors have been developed. In melanoma, there is some evidence that increased RAD21 expression correlates with sensitivity to CHK1 inhibitors (CHK1i). Therefore, we tested the hypothesis that MYC and RAD21 act as predictive biomarkers of therapeutic response to BETi (OTX-015) and CHK1i (LY2606368 and CCT245737), respectively, in pediatric OS cell lines and OS patient-derived xenografts (PDXs) established from Riley Hospital patients (TT1, TT2).

TT1 and TT2 were developed from the same patient at different therapeutic phases and harbor MYC-RAD21 CNVs based on whole genome sequencing (WGS). WGS of two PDX passages (MP1 and MP2) from both TT1 and TT2 was performed to compare and evaluate the stability of the molecular signature to the original tumor. In both TT1 PDX passages PTEN loss was observed which was not present in the original tumor. In TT2, CCND3 and NCOR1 CNVs were observed in the original tumor and maintained in both MP1 and MP2. These PDX models provide innovative opportunities to link the molecular signature and past patient treatment histories with potency of response to BETi and CHK1i.

Established pediatric OS cell lines (Saos2, Saos-LM7, G292, MG63, U2OS) and TT2 PDX-derived cell line as well as TT2 3D sarcospheres co-express MYC and RAD21. BETi and CHK1i treatment as single agents demonstrated potent growth inhibition by methylene blue assay in all establised OS cell lines. While the TT2 PDX-derived cell line was highly sensitive to single-agent BETi and CHK1i, the TT2 PDX-derived 3D sarcospheres were only sensitive to CHK1i. The predictive value of these in-vitro findings will be evaluated in an in vivo TT2 PDX model. Notably BETi and CHK1i dual-targeting showed synergistic growth inhibition in Saos2 and Saos-LM7 lines that express high levels of Cyclin E2, a promoter of G1/S progression. In contrast, dual targeting was additive-to-antagonistic in OS cells with low Cyclin E2 expression. Bromodomain, BRD4, can indirectly regulate the expression of Cyclin E2 by activating E2F via direct regulation of Cyclin D-pRB pathway. Studies are in progress to evaluate this mechanistic link and determine why some OS cells respond and others do not to dual targeting of MYC and CHK1 in both 2D and 3D in vitro models.

Translational/Clinical Research ***Faculty***

POSTER #76

EXPRESSION OF CORNULIN IN ORAL PREMALIGNANT LESIONS

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Objective: To evaluate expression of cornulin in oral mucosa as an adjunct to histopathologic grading of oral epithelial dysplasia (OED).

Study design: Cornulin expression was assessed in normal oral mucosa (NOM), low-grade (LD) and high-grade (HD) OED and oral squamous cell carcinoma (OSCC) using immunohistochemistry (IHC). Photomicrographs were evaluated with Aperio Imagescope using the positive-pixel-counting algorithm. A histo-score (H-score) was calculated based on the staining intensity and the percentage of positive cells. Intra-rater reliability for H-score and %-staining was determined by calculating interclass correlation coefficients (ICCs). Mean differences in H-scores and %-staining values were each analyzed using an analysis of variance and Tukey post hoc procedure.

Results: Cornulin expression progressively diminished with increasing grades of dysplasia and OSCC. ICCs for H-score and %-staining were each >0.99. Except for OSCC vs HD, all other pairwise comparisons were statistically significant ($P<0.0001$) for H-score and %-staining.

Conclusion: Cornulin expression differentiated between low-grade and high-grade oral epithelial dysplasia, making it a potential adjunct for grading oral OEDs and a potential biomarker for risk of lesion progression. Longitudinal studies evaluating risk stratification based on cornulin expression may be warranted.

Translational/Clinical Research ***Faculty***

POSTER #77

DISCRIMINATION BETWEEN TRANSLOCATION AND CLEAR CELL RENAL CELL CARCINOMA BY COMPUTATIONAL ANALYSIS OF H&E PATHOLOGY IMAGES

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Motivation: Translocation renal cell carcinoma (TRCC) is a relatively new subtype of renal cell carcinoma (RCC) with chromosomal translocations involving transcription factors *TFE3* (Xp11.2) or, less frequently, *TFEB* (6p21). TRCC represents 1% to 5% of all cases of RCC, with higher frequency in young patients. The Xp11 translocation RCCs frequently present clear cells morphology, which makes it very difficult for pathologists to distinguish TRCC from clear cell RCC using routine H&E stained images. Therefore, in clinical practice the differential diagnosis is carried out with techniques that are more challenging —immunohistochemical (IHC) array and/or fluorescence in situ hybridization (FISH) assay [1]. In this study, we investigate that whether computational analysis of H&E images is able to help pathologists discriminate between TRCC and clear cell RCC.

Methods: We collect the whole-slide H&E pathology images from 20 patients with Xp11 TRCC and 28 patients with clear cell RCC, respectively. TRCCs are diagnosed by IHC and further confirmed by FISH. A total of 150 image features, charactering the size, shape, color, and density of cells, are extracted from the whole-slide images [2]. The Mann-Whitney U test is used to find image features showing significant difference between the two RCC subtypes. The false discovery rate adjustment is applied for multiple testing correction. In addition, we split the dataset into training and test sets. We build a lasso-regularized logistic regression model on the training set and evaluate the model on the untouched test set. The performance is measured by the area under receiver operating characteristic curve (AUC).

Results: The Mann-Whitney U-test discovers 30 out of 150 image features that significantly differ between TRCC and clear cell RCC after multiple testing correction, such as area_bin1, area_std, ratio_1, and ratio_bin3. In the obtained lasso-regularized logistic regression model, six features are selected (gMean_std, area_bin5, gMean_kurtosis, rMean_bin6, rMean_skewness, ratio_bin3). The classification model on the test set achieves an AUC of 0.829.

Conclusions: Computational pathology allows systematic characterization of the diverse structures present in pathology images and capture of subtle differences between images. These subtle differences confer valuable information for differential diagnosis and are unlikely to be perceived by pathologist's eyes. Our study demonstrates that computation analysis of H&E pathology images can assist pathologists in discriminating TRCC from clear cell RCC.

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Translational/Clinical Research ***Faculty***

POSTER #78

ADVANCING GLIOBLASTOMA (GBM) DRUG REGIMEN DEVELOPMENT TO SUPPORT COMBINATION THERAPY THROUGH INTEGRATED PKPD MODELING AND SIMULATION-BASED PREDICTIONS

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Despite advancements in therapies, such as surgery, irradiation (IR) and chemotherapy, outcome for patients suffering from glioblastoma remains fatal; the median survival rate is only about 15 months. Even with novel therapeutic targets, networks and signaling pathways being discovered, monotherapy with such agents targeting such pathways has been disappointing in clinical trials. Poor prognosis for GBM can be attributed to several factors, including failure of drugs to cross the blood-brain-barrier (BBB), tumor heterogeneity, metastasis and angiogenesis. Development of tumor resistance, particularly to temozolomide (TMZ), creates a substantial clinical challenge.

The primary focus of our work is to rationally develop novel combination therapies and dose regimens that mitigate resistance development. Specifically, our aim is to combine TMZ with small molecule inhibitors that are either currently in clinical trials or are approved drugs for other cancer types, and which target the disease at various resistance signaling pathways that are induced in response to TMZ monotherapy. To accomplish this objective, an integrated PKPD modeling approach is used. The approach is largely based on the work of Cardilin, et al, 2018. A PK model for each drug is first defined. This is subsequently linked to a PD model description of tumor growth dynamics in the presence of a single drug or combinations of drugs. A key outcome of these combined PKPD models are tumor static concentration (TSC) curves of dual or triple combination drug regimens that identify combination drug exposures predicted to arrest tumor growth. This approach has been applied to TMZ in combination with abemaciclib (a dual CDK4/6 small molecule inhibitor) based on data from a published study evaluating abemaciclib efficacy in combination with TMZ in a glioblastoma xenograft model (Raub, et al, 2015).

A PKPD model was developed to predict tumor growth kinetics for TMZ and abemaciclib monotherapy, as well as combination therapy. Population PK models in immune deficient NSG mice for temozolomide and abemaciclib were developed based on data obtained from original and published studies. Subsequently, the PK model was linked to tumor volume data obtained from U87-MG GBM subcutaneous xenografts, again using both original data as well as data from the Raub, et al, 2015 study. Model parameters quantifying tumor volume dynamics were precisely estimated (coefficient of variation < 30%). The developed PKPD model was used to calculate plasma concentrations of TMZ and abemaciclib that would arrest tumor growth, as well as combinations of concentrations of the two drugs that would accomplish the same endpoint. This so-called TSC curve for the TMZ and abemaciclib combination pair evidenced an additive effect of the two agents when administered together. These results will be presented. In addition, results from on-going PKPD studies of TMZ in combination with two other small molecule inhibitors, RG7388, an MDM2 inhibitor, and GDC0068, an AKT inhibitor, will also be presented.

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

POSTER #79

IMPACT OF SARCOPENIA ON POST – OPERATIVE OUTCOMES OF AUTOLOGOUS FREE TISSUE TRANSFER IN HEAD AND NECK RECONSTRUCTION

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Objective: To determine the clinical impact of sarcopenia on post-operative outcomes in head and neck cancer patients undergoing reconstruction with autologous free tissue transfer.

Study Design: Retrospective case-control study

Setting: Tertiary referral center

Patients: One hundred and twenty nine (129) patients with head and neck cancer who received oncologic resection followed by reconstructive autologous free tissue transfer between January 2014 and December 2018 with adequate images were included in the study.

Methods: Digital Imaging and Communication in Medicine (DICOM) images of preoperative abdominal CT scans were reviewed at the third lumbar (L3) vertebra to calculate skeletal muscle cross-sectional area (CSA, cm^2) using SliceOmatic software v5.0 (TomoVision, Magog, Canada). Skeletal muscle Hounsfield Units (HU) of -29 to +150 were used to isolate skeletal muscles using thresholding techniques. Skeletal muscle index (SMI, cm^2/m^2) was calculated by normalizing CSA to patient height in meters squared. Sarcopenia at L3 was defined as SMI = 41.6 cm^2/m^2 for males and = 32.0 cm^2/m^2 for females, as previously reported.

Main Outcome Measures: Primary outcome measures included (1) prevalence of sarcopenia in head and neck cancer patients, (2) incidence of post-operative complications in sarcopenic patients (as listed by the American College of Surgeons National Surveillance and Quality Improvement Program [NSQIP]), (3) incidence of flap-specific complications in sarcopenic cancer patients.

Results: Of the 129 patients who met inclusion criteria, 35 patients (27.1%) were determined to have pre-operative sarcopenia. The sarcopenic group was older (63 vs 58 years, $p = 0.017$), had lower BMI (21.2 vs. 27.2, $p<0.001$), had greater incidence of alcohol abuse (55.3% vs. 23.1%, OR = 4.11, $p < 0.001$), and had lower ECOG scores (1.03 vs 0.51, $p=0.008$). Intra-operatively, sarcopenic patients were found to have higher rates of blood transfusions (63.8% vs. 29.8%, $p < 0.001$). Post-operatively, sarcopenic patients had higher rates of pneumonia ($p < 0.01$), prolonged ventilation ($p < 0.01$), venous thromboembolism ($p < 0.01$), delirium ($p < 0.01$), fistula ($p < 0.05$) and longer ICU stays ($p < 0.05$). Sarcopenic patients were ultimately found to have higher overall rates of NSQIP complications ($p < 0.001$) and overall flap-specific complications ($p < 0.01$).

Conclusions: Our study characterizes the prognostic value of sarcopenia for outcomes of autologous free tissue transfer in head and neck reconstruction. Sarcopenia was found to be highly prevalent in our patient population and was a positive predictor of postoperative complications.

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

POSTER #80

TRANSCRIPTOMIC PROFILING OF OVARIAN CLEAR CELL ADENOCARCINOMA IN WOMEN WITH CONCURRENT ENDOMETRIOSIS

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Endometriosis is a chronic inflammatory disease whereby endometrial-like tissue grows outside the uterus. Endometriosis is a significant risk factor for ovarian cancer. Women with endometriosis are at three-fold increased risk of ovarian clear cell adenocarcinoma than women without endometriosis. While clear cell carcinomas are considered a rare histologic subtype, responsible for only 10% of epithelial ovarian cancers, they carry a worse prognosis than the more common high-grade serous carcinomas of similar stage. This poor prognosis likely stems from poor response of clear cell carcinomas to standard-of-care platinum-based therapies. The endometriotic tumor microenvironment may play a role in ovarian cancer biology. Our previous bioinformatic analysis showed that ovarian endometrioid adenocarcinoma from women with concurrent endometriosis was molecularly distinct from ovarian endometrioid adenocarcinoma from women without endometriosis. Out of these molecularly distinct pathways, TGFß signaling was significantly upregulated in tumors with endometriosis compared to endometriosis. Because each histologic subtype of epithelial ovarian cancer is molecularly unique, we procured clear cell carcinoma and endometriosis tissues and performed next-generation sequencing. Pathology-confirmed clear cell carcinomas from women with concurrent endometriosis and endometriomas, large endometriotic cysts of the ovary were compared at both the mRNA and miRNA levels. Analysis of mRNA transcripts identified 4799 differentially expressed genes (2223 up, 2576 down; $P<0.01$; log2fold change >1 or <-1). Small RNA sequencing analysis identified 66 differentially expressed miRNAs (19 up, 47 down, $P = 0.05$; fold change >1.2 or <-1.2). MiR-10a was 11-fold upregulated in clear cell carcinoma with concurrent endometriosis, and Ingenuity Pathway Analysis indicated that miR-10a was a significant upstream regulatory molecule (P=7.6e-3). Integrated analysis of dysregulated genes and dysregulated miRNA molecules with *in silico* miRNA target prediction algorithms predicted 1908 miRNA-target genes reciprocally dysregulated (1009 up, 899 down). MiR-10a is predicted to affect 117-target genes dysregulated. Functional annotation of these 117 miR-10a target-genes showed enrichment in inflammation, proliferation, and transcription factor regulation. Overall, this data indicates miR-10a as a possible driver and therapeutic target in endometriosis-associated-ovarian clear cell adenocarcinoma.

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

POSTER #81

MODELLING TAXANE-INDUCED PERIPHERAL NEUROPATHY IN HUMAN IPSC-DERIVED NEURONS

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Taxanes are common first-line chemotherapeutic agents for various cancers, but are not without toxicity. Taxane-induced peripheral neuropathy (TIPN) is one of the most important survivorship issues for patients receiving taxanes therapy.However, the mechanism underlying TIPN remains enigmatic. While it may be scientifically appealing to use primary tissue for investigating the mechanisms of TIPN, procuring nerves from patients is not realistically feasible, as nerve biopsies are painful and may result in permanent damage. Recently developed techniques to generate human-derived neurons from induced pluripotent stem cells (iPSCs) has provided a promising alternative model for exploring the mechanisms of TIPN. iPSCs are undifferentiated and endlessly dividing cells that can be generated from a patient's somatic cells, such as peripheral blood mononuclear cells (PBMCs). Reprogramming of PBMCs entails the alteration of DNA methylation patterns and genomic transcriptional activity, reverting these cells into an embryonic state, which enables them to be further induced into various cell lineages. This technique has become pivotal in the advancement of basic and translational biomedical research. However, there is evidence that iPSCs may retain trace epigenetic memories of their donor tissues, which raises concern on whether iPSC-derived lines reflect primary cells with appropriate fidelity.

In this study, we have successfully reprogrammed PBMCs into iPSC-derived sensory neurons and characterized the neuronal population we expect to see using a differentiation protocol that bypasses the neural progenitor stage and have found that these recapitulate mature neuronal phenotypes as follows. Flow cytometry analysis data demonstrate that reprogrammed iPSCs have acquired pluripotency after 12 passages (p12) and are prepared for induction to neurons. Electrophysiology and calcitonin gene-related peptide (CGRP) release data support functionality and provide insight into the timing for which downstream assays can be performed (week 4-post induction). Finally, we are currently comparing the epigenetic signatures of iPSC-derived neurons to that of isogenic primary nerves through a prospective protocol of neurons from patients undergoing amputation.

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

POSTER #82

APLIDIN IS A NOVEL MARINE-DERIVED AGENT THAT INHIBITS BONE RESORPTION AND PREVENTS MM-INDUCED BONE DISEASE

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Multiple Myeloma (MM) is a hematological malignancy characterized by exacerbated activity of osteoclasts and the consequent development of osteolytic lesions that weaken the bone and increase the risk of fracture. Aplidin is a novel marine derived chemotherapeutic agent with anti-MM activity. We recently found in vitro that Aplidin also decreases osteoclast precursor numbers and differentiation, and reduces mature osteoclast number and resorption activity, suggesting that Aplidin exhibits anti-resorptive effects in the skeleton. Here we report the in vivo effects of Aplidin on MM-induced bone disease (MMBD) using an immunodeficient xenograft mouse model of established MM. 8-wk-old SCID mice were intratibially inoculated with human MM1.S MM cells, or saline as control. After 5 wks, MM-injected mice exhibited detectable levels of the serum tumor biomarker human ¿ Light Chain (h¿LC), whereas this marker was undetectable in the serum of saline-injected mice. Control and MM-injected mice were then randomized by MM tumor burden and bone mineral density to subgroups receiving either injections of vehicle or Aplidin (0.1mg/kg/day). After 2-wks of treatment, serum h¿LC was similar in mMM-injected mice receiving vehicle or Aplidin. MM-injected mice displayed extensive MMBD as determined by a 50% decrease in cancellous bone/volume (BV/TV), trabecular number (Tb.N.), trabecular thickness (Tb.Th.), and increase in trabecular separation (Tb.Sp.) compared to control mice receiving vehicle injections. In contrast, treatment with Aplidin fully protected MM-injected mice from MMBD, with BV/TV, Tb.N, Tb.Th, and Tb.Sp. values undistinguishable from those seen in control mice receiving Aplidin. Further, Aplidin significantly reduced the serum levels of the bone resorption marker CTX by 30% and the bone formation marker P1NP by 75% in both saline- and MM-injected mice. Taken together, these results demonstrate that Aplidin has potent anti-resorptive effects and prevents MMBD. These findings provide the framework for combining Aplidin with other anti-MM agents to simultaneously decrease tumor growth and prevent bone destruction.

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

POSTER #83

HIGHLY ROBUST MODEL OF TRANSCRIPTION REGULATOR ACTIVITY PREDICTS BREAST CANCER PROGNOSIS

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Abstract

While early clinical diagnosis of breast cancer has proven effective, the development of effective molecular indicators can improve treatments and predict diagnostic outcomes. The objective of this study was to identify transcriptional regulatory networks to understand breast cancer development mechanisms and incorporate this information into a model for predicting clinical outcomes. Gene expression profiles from 1097 breast cancer patients were retrieved from The Cancer Genome Atlas (TCGA). Breast cancer-specific transcription regulatory information was identified by considering the binding site information from ENCODE and the top co-expressed targets in TCGA using a nonlinear approach. We used this information to build a multi-regulator linear model to predict breast cancer patient survival. This model was validated in more than 5000 breast cancer patients from the Gene Expression Omnibus (GEO) databases. Our findings demonstrate that transcriptional regulator activities can predict patient survival. This finding provides additional biological insights into the mechanisms of breast cancer progression.

Keywords: Breast cancer, transcription regulators, prognostic model

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

POSTER #84

CANCER ASSOCIATED FIBROBLASTS PROMOTE OVARIAN CANCER CHEMORESISTANCE BY INDUCING CANCER STEM CELLS THROUGH WNT SIGNALING

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Ovarian cancer is the most lethal gynecologic malignancy and the 5th leading cause of cancer related deaths among women in the USA. Most patients eventually succumb to chemoresistant disease and the purpose of this study is to understand the mechanism of development of chemoresistance and disease relapse. Cancer stem cells (CSCs) consist of a small subpopulation in the tumor that are resistant to cytotoxic chemotherapy and cause relapse. The tumor microenvironment can potentially provide an optimal cancer stem cell niche for cancer stem cell growth and maintenance. Cancer associated fibroblasts (CAFs) are one of the main constituents of the tumor microenvironment in ovarian tumors, promoting tumor progression and chemoresistance. We have studied the potential role of CAFs in maintaining CSC population and enhancing chemoresistance with an objective to develop effective approaches to overcome chemoresistance and tumor relapse. Co-culture of high grade serous ovarian cancer cells with CAFs resulted in increased resistance to carboplatin. ALDH1A1 is a well-established marker for ovarian cancer CSCs and the ALDH$^+$ population was significantly increased upon co-culture with CAFs. Similarly, CAFs also enhanced spheroid formation of ovarian cancer cells seeded in ultralow adhesion plates in CSC medium. Interestingly, co-culturing ALDH$^-$ ovarian cancer cells with CAFs resulted in the induction of ALDH$^+$ cells within 6 days. Analysis of the signaling pathways activated in ovarian cancer CSCs and the gene expression profiles of ovarian cancer CAFs indicated the potential role of Wnt signaling in the productive cross-talk between CAFs and ovarian cancer CSCs. Treatment with Wnt inhibitors abrogated the induction of CSCs by CAFs. By selectively silencing porcupine, a protein involved in Wnt ligand lipidation and secretion, we further confirmed that CAF derived Wnts are responsible for the induction of CSC. Studies are ongoing to identify the specific Wnt ligand involved in the cross-talk and downstream pathways activated during CSC induction and maintenance by CAFs. Our results indicate that CAF-derived Wnt ligands are instrumental in ovarian cancer CSC growth and maintenance. In the long term, our studies will broaden the understanding of CSC maintenance by the tumor microenvironment and contribute towards the development of novel therapeutic approaches to prevent ovarian cancer chemoresistance and relapse.

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

POSTER #85

DISRUPTION OF NOTCH SIGNALING TARGETED TO THE MYELOMA BONE MARROW MICROENVIRONMENT SIMULTANEOUSLY INHIBITS TUMOR GROWTH AND PREVENTS BONE LOSS WITHOUT INDUCING GUT TOXICITY

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Communication between multiple myeloma (MM) cells and cells of the bone/bone marrow microenvironment via Notch signaling promotes MM growth and survival and stimulates bone resorption. Systemic inhibition of Notch, using g-secretase inhibitors (GSI), decreases MM growth and reduces bone destruction; however, the clinical use of GSI is limited due to severe gut toxicity.

To circumvent GSI side effects, we designed and generated a novel bone specific Notch inhibitor (BT-GSI) by conjugating GSI-XII to a targeting molecule (BT) with high bone affinity using an acid hydrolyzable linker. *In vitro*, BT-GSI was inactive unless pre-incubated at low pH, and exhibited equal inhibition of Notch target genes in MM cells as unconjugated GSI. *Ex vivo*, BT-GSI decreased the expression of Notch target genes and reduced MM growth in bone organ cultures that reproduce acidic conditions in the MM-bone microenvironment. Next, we examined *in vivo* the impact of BT-GSI on Notch signaling in bone, MM growth, and bone disease in a preclinical model of established MM. 8-wk-old immunodeficient mice were injected intratibially with 10^5 JJN3 human MM (hMM) cells or saline. hMM injected mice exhibited detectable serum levels of the tumor biomarker human K-light chain (40 ng/mL) and visible osteolytic disease (osteolytic area 1.7 mm^2) 3 weeks after hMM inoculation. Then, hMM-injected mice were randomized based on tumor levels to two subgroups to receive either BT-GSI (10mg/kg/3x/wk) or vehicle (DMSO) for 3 weeks. Saline-injected mice received vehicle injections. BT-GSI selectively decreased Notch gene expression in bone, but had no effect in the brain or gut. Further, BT-GSI did not increase the expression of Adipsin in intestinal tissue, a marker of gut toxicity, nor showed visual evidence of gut toxicity at necropsy. MM-bearing mice treated with BT-GSI exhibited a 45% decrease in tumor burden (168 vs 254 ng/mL human K-light chain) and 50% less osteolytic area (4.4 vs 10.2 mm^2) compared to vehicle treated mice. Moreover, BT-GSI decreased the serum levels of the bone resorption marker CTX by 30%, but did not affect the serum levels of the bone formation marker P1NP. Importantly, equimolar administration of the unconjugated BT agent did not alter MM growth nor prevented bone loss in mice with established MM.

In conclusion, these results show that bone-targeted Notch inhibition reduces MM growth and preserves bone mass in mice with established MM. Because BT-GSI shows bone specific Notch inhibition and lacks gut toxicity, it should circumvent the deleterious side effects that limit GSI use in patients. Thus, BT-GSI is a promising approach to inhibit MM growth and to prevent bone loss in MM patients.

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

POSTER #86

INHIBITION OF FGFR1 IN BREAST CANCER METASTASIS

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Metastatic breast cancer is the most advanced stage of breast cancer. However, our understanding of the molecular mechanisms which drive metastatic breast cancer remains incomplete. Epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET) promote tumor invasion and metastasis. Previously, it has been reported that fibroblast growth receptor 1 (FGFR1) plays a key role during EMT: MET cycle. Therefore, optimizing FGFR inhibitors is crucial for therapeutic targeting of late-stage breast cancer. Here, we examined the efficacies of three FGFR kinase inhibitors, AZD4547, JNJ-42756493 and FIIN4, in the 4TO7 cell line both in vitro and in vivo. The 4TO7 cell line is a syngeneic model of systemic dormancy. Mammary fat pad engraftment of the 4T07 cells onto immune competent Balb/C mice results in systemic dissemination, but no macroscopic metastasis. In contrast, the 4T07 cells can escape cellular dormancy in 3D culture and form robust pulmonary tumors in mice upon tail vein inoculation. Furthermore, we developed a 3D culture approach that combines tumor spheroid formation in a non-adherent round bottom dish followed by placement on a bed of matrix. Our in vitro results demonstrate that both JNJ-42756493 and FIIN4 have more potent anti- proliferative activities in 3D culture and longer residence time as compared to AZD4547. In vivo, inhibition of FGFR is highly effective against 4TO7 tumors in the pulmonary microenvironment. Importantly, FIIN4 showed the least toxicity. This result suggested that covalent inhibition of FGFR is a promising cancer therapy for patients with metastatic breast cancer. Besides, metastasis comes from disseminated tumor cells (DTCs) which can enter dormancy and escape therapies after primary tumor removal. Moreover, covalent inhibition of FGFR1 targeted metastatic breast cancer. Therefore, we are wondering whether inhibition FGFR1 can target dormant breast cancer cells. To investigate this question, we tested different FGFR1 inhibitors in D2.OR cells which dormancy depends on soft matrices. The data showed that FGF2 which is the ligand of FGFR1 could break the D2.OR dormancy in the 3D environment. Inhibition of FGFR kinase activity abolished the increased cell viabilities which stimulated by FGF2 in the 3D environment.

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

POSTER #87

A CASE OF GLUCOSE PHOSPHATE ISOMERASE DEFICIENCY WITH DEVELOPMENT OF SECONDARY HEMOCHROMATOSIS, CIRRHOSIS, AND LIVER FAILURE

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Glucose-6-Phosphate Isomerase deficiency is a rare disorder characterized by a GPI gene mutation with either homozygous or compound heterozygous inheritance. Only fifty cases have been described since its discovery in 1968(1). A 62yoWF with a PMH of Glucose-6-Phosphate-Isomerase deficiency, subsequent iron overload as a complication requiring intermittent chelation therapy, and liver cirrhosis was admitted to the MICU. Symptoms included two days of diarrhea, hypoglycemia, and a BP of 69/43mmHg. Levophed, Vancomycin, Zosyn, and Sodium Bicarbonate were initiated. Initial labs included: Creatinine 7.3mg/dL (baseline 1.4mg/dL), ammonia 113umol/L, total bilirubin 45.0mg/dL (baseline 5.0mg/dL), alkaline phosphatase 145units/L, AST 194units/L, ALT 282units/L, albumin 2.2GM/dL, INR 2.2, and lactic acid 3.9mmol/L. She was found to be in decompensated liver cirrhosis with a MELD score of 45 and Child Pugh Class C. Lactulose, Rifampin, Zinc, and Vitamin K were initiated as well. Hematology was consulted due to worsening total bilirubin (62.2mg/dL). Peripheral smear displayed irregularly folded RBC's and several hypochromic macrocytes. With aggressive treatment, clinical improvements were made. Unfortunately, the patient developed seizures. CT head revealed intraparenchymal hemorrhage with fluid level, the cause believed to be secondary to septic emboli. Due to the poor prognosis, the patient was made comfort care and passed away shortly thereafter. There has only been one other documented case of a patient with this deficiency developing iron overload(2). However, our patient is the first to have documented cirrhosis and liver failure associated with it. One possible explanation as to the cause could be related to hepcidin levels with elevated levels causing an inability to use stored iron like in anemia of chronic disease. Another possibility is hepcidin deficiency similar to what has been described in B-thalassemia intermedia, with increased iron absorption and iron overload(3). No records of HFE testing were available, and she rarely required transfusions throughout her life according to outpatient hematology notes, so it is unlikely these caused the high ferritin levels. There are currently no guidelines on iron chelation therapy for persons with this disease who develop iron overload. Providers should discuss the various chelation therapies available and choose therapy based on individual patient factors until more data can be gained to prevent long term complications of this disorder as seen in the case above.

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

POSTER #88

IDENTIFYING FUNCTIONAL DRIVERS OF IMMUNOTHERAPY RESISTANCE

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Castration resistant prostate cancer (CRPC) is one of the leading causes of death in men in the United States, with a 5-year survival rate at 28%. Immune checkpoint blockade (ICB) is a novel therapeutic strategy based on restoring the tumoricidal activity of cytotoxic T-lymphocytes (CTLs). ICB therapy has produced some spectacular results in the treatment of metastatic melanoma but shows almost no effect in CRPC. Two of the most commonly used ICB drugs target programmed death-1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). PD-1 is expressed on CTLs and if it binds its ligand PD-L1 the CTLs will become inactivated. CTLA-4 is a protein expressed on activated T-lymphocytes that attenuates T-cell activation by removing the costimulatory ligand for the CD28 receptor from the immunological synapse.

Through serial implantation of tumors derived from a spontaneous prostate tumor with a P53/Pten/Smad4 deletion, we have generated sublines that are either resistant to PD-1 therapy, CTLA-4 therapy, or both drugs. Subsequent RNA sequencing showed significant transcriptomic changes associated with drug resistance. We subsequently perturbed transcriptomic regulation by treating the cells with vorinostat, a pan-histone deactetylase inhibitor. This resulted in significant changes in the transcriptome. One of the transcriptional changes involves a strong upregulation of genes involved in antigen presentation.

In our *in vivo* model systems, treatment of ICB resistant tumors with a combination of ICB drugs and vorinostat resulted in an objective response in 80% of animals. The combination therapy effectiveness is dependent on CD8 T-cells. Cytometric analysis using unbiased machine learning approaches have revealed that vorinostat treatment attenuates ICB-induced FoxP3+ regulatory T-cell increases in the tumor immune microenvironment. Combining ICB with vorinostat treatment decreases M2-macrophage polarization, and it increases tumor infiltration of CD4 T-cells and conventional dendritic cells.

Our goal is to gain a mechanistic understanding of ICB resistance in CRPC, so that we can identify potential drug targets for combination treatment.

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

POSTER #89

ISSUES IN TISSUE PROCESSING AND IDENTIFICATION FOR GENOMIC SEQUENCING IN RILEY HOSPITAL FOR CHILDREN PEDIATRIC SARCOMA PATIENTS

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Pediatric sarcomas make up 13% of cancers experienced by patients under the age of 20. The overall five-year survival rate for pediatric sarcomas is around 60%. A large percentage of pediatric sarcomas are Ewing sarcoma, rhabdomyosarcoma, synovial sarcoma, osteosarcoma, and others. Biomarker studies are being conducted to understand the underlying mechanisms and advance targeted therapies. Studies have turned to focus on genomic sequencing at the DNA level. The Precision Genomics Program at Riley Children's Hospital at Indiana University Health was created with this focus in mind. The program uses genetic testing to identify DNA, RNA and proteins that can be targeted with various treatments. Identification of high quality tissue samples is imperative for this endeavor. Slides were reviewed and followed pathology guidelines that were created by the Genotype-Tissue Expression Project (GTEX - normal tissue) and NCI Total Cancer Genome Atlas (TCGA - tumor) in 2012. The guidelines are as follows: H&E slides were QC'd for % tumor (>60%), necrosis (<20%), stroma (<10%), and inflammation (<10%). Additionally, the tissues were analyzed for number of viable cells for the genomics study (>400). Decalcified slides were removed from this genomics study. Out of the 79 deceased cases that were analyzed, 7 were decalcified and not viable for genomic analysis. In 49 living cases 16 were decalcified and not viable for the study. Samples containing limited tissue such as fine needle aspirations and blood smears were removed from the study in addition to hemorrhagic and necrotic tissue.

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

POSTER #90

HOTAIR FUNCTIONALITY AND REGULATION IN OVARIAN CANCER STEM CELLS

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Abstract:

Ovarian cancer (OC) is the fifth leading cause of cancer-related death among American women. Persistence of OC stem cells (OCSCs) is believed to contribute to resistance to platinum-based chemotherapy and disease relapse. We have previously shown that epigenetic changes in OCSCs play a role in post-therapy OCSC persistence and demonstrated that is it possible to target OCSC using epigenetic therapies. HOXC transcript antisense RNA (HOTAIR) has been shown to be associated with chemoresistance and overexpressed in many types of cancers, including high-grade serous OCs (HGSOCs). HOTAIR interacts with Polycomb Repressive Complex 2 (PRC2) and due to its histone methyltransferases activity plays a key role in chromatin remodeling. Because HOTAIR is a known epigenetic regulator of differentiation and developmental genes in OC and other cancers, we hypothesize that HOTAIR is a key epigenetic regulator in OCSCs and therapeutically targeting HOTAIR in OCSCs will prevent tumor relapse.The goal of this study is to understand the role and the underlying mechanistic function of HOTAIR in regulating OCSCs. Aldehyde dehydrogenase (ALDH) activity and FACS was used to separate OCSCs from non-OCSCs in a panel of HGSOC cell lines (OVCAR3, CAOV3, OVCAR5, Kuramochi, COV362). Quantitative RT-PCR analysis revealed that HOTAIR was overexpressed in OCSC cells compared to non-OCSC cells. Knockout of HOTAIR using CRISPR-Cas9 system significantly decreased OCSC population and stemness-related phenotypes, including spheroid formation and colony formation ability. Overexpression of HOTAIR in OC cells significantly increased these stem-like characteristics. Furthermore, targeting HOTAIR using peptide nucleic acid (PNA), which blocks interaction with Enhancer of Zeste Homologue 2 (EZH2), significantly decreased the OCSC population, indicating HOTAIR functions through EZH2 in regulating OCSCs. Combining the HOTAIR targeting PNA with other epigenetic inhibitors, including the hypomethylating agent guadecitabine and the EZH2 inhibitor GSK503, significantly decreased proliferation and colony formation ability of HGSOC cells. We suggest that a better understanding of HOTAIR will facilitate identifying epigenomic alterations and chromatin landscape that contributes to OCSC phenotypes. Targeting HOTAIR in combination with epigenetic therapies may represent a therapeutic strategy to prevent tumor relapse.

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

POSTER #91

GENE CO-EXPRESSION NETWORK AND COPY NUMBER VARIATION ANALYSES IDENTIFY TRANSCRIPTION FACTORS ASSOCIATED WITH MULTIPLE MYELOMA PROGRESSION

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Multiple myeloma (MM) is the second most common blood cancer and arises from malignant bone marrow plasma cells. MM is preceded by two clinical precursor stages of disease, termed monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM). However, the genomics of progression during the precursor stages is not well understood. We used a gene co-expression network analysis pipeline to identify transcription factors of interest associated with myeloma progression in two publicly available datasets and validated them by copy number variation analysis in a third public dataset. First, we identified co-expressed gene modules in two gene expression datasets each containing three conditions: normal, MGUS, and SMM, and then assessed each module for gene co-expression specificity to a single condition. These condition-specific gene modules were used to identify enriched transcription factors. Transcription factors were evaluated for differential expression and copy number variation between normal and MM precursors. Our pipeline identified functionally associated transcription factors that were differentially expressed or showed significant changes in copy number. Overall, we identified four genes of interest (*MAX*, *TCF4*, *ZNF148*, and *ZNF281*), supported by the literature, that contribute to our understanding of MM initiation and progression.

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

POSTER #92

THE ROLE OF PSA MONITORING AFTER HOLMIUM LASER ENUCLEATION OF THE PROSTATE (HOLEP)

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Introduction*:* The utilization of PSA for the diagnosis of prostate cancer has been recently challenged due to poor sensitivity as a number of conditions can contribute to an elevated PSA with BPH most notable among them. Because HoLEP patients have the entirety of their transition zone removed, we hypothesized that PSA changes after surgery would be a sensitive measure of prostate cancer risk.

Methods: A retrospective review of an IRB database identified 2619 HoLEP surgeries performed at Indiana University from 2008 to 2018. 55 post-HoLEP biopsies were recorded. Demographics, PSA, prostate volume, and oncologic details were analyzed. PSA density was calculated using post-HoLEP PSA and post-operative prostate volume.

Results*:* A total of 55 patients underwent "for cause" transrectal ultrasound prostate biopsy following HoLEP at a median time frame of 18.5 months after surgery. The mean PSA prior to biopsy for these patients was 6.32 ng/mL. Cancer was identified in over 90% (50/55) of those biopsied. In the HoLEP cohort, 94% of patients with a PSA above 1 at the time of biopsy were found to have prostate cancer. Amongst analyzed patients, a PSA of 5.8 of higher was universally associated with clinically significant disease.PSA density above 0.1 $ng/mL^2$was associated with over a 95% risk of prostate cancer and 88% risk of clinically significant prostate cancer. As PSA density rose above 0.2 ng/mL^2, men in our cohort harbored a 100% probability of clinically significant prostate cancer

Conclusions: Post HoLEP PSA and PSA density are extremely sensitive measures for selecting patients at risk for prostate cancer. Gross PSA threshold for biopsy should be lower than for non-HoLEP patients. HoLEP patients with PSA density of 0.1 or higher have a high likelihood of harboring clinically significant prostate cancer and should undergo prostate biopsy if feasible. Referring urologists and primary care physicians should be made aware of the significant risk shifts when performing routine PSA screening in patients with prior HoLEP surgery.

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

POSTER #93

CUTANEOUS METASTASIS IN A PATIENT WITH MUIR-TORRE SYNDROME: A CASE REPORT AND REVIEW OF THE LITERATURE

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Muir-Torre syndrome (MTS) is a variant of Lynch syndrome in which patients develop sebaceous neoplasms and internal malignancies. We describe the clinical features of a 46-year-old man with MTS and a remote history of sebaceous carcinoma who presented to the Mohs micrographic surgery unit for treatment of chin and neck lesions after recently being diagnosed with gastric adenocarcinoma. Preoperative diagnosis was not finalized, as fine needle aspiration could not differentiate between sebaceous carcinoma and cutaneous metastasis. Final pathology confirmed that both lesions were cutaneous metastases from gastric adenocarcinoma.

We conducted a literature review by querying the PubMed database with various combinations of the key words: adenocarcinoma, cancer, cutaneous, gastric, metastasis, Muir-Torre syndrome, skin, and sebaceous. The papers generated by the search and their references were reviewed. The literature review revealed only one previously reported case of cutaneous metastasis in patients with MTS. To the best of our knowledge, we report the first case of cutaneous metastasis from gastric adenocarcinoma in a patient with MTS. Cutaneous metastasis from gastric adenocarcinoma is rare but should be considered in the differential diagnosis of new skin lesions in patients with known underlying malignancy.

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

POSTER #94

LOCALIZED BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM TREATED WITH INTRALESIONAL TRIAMCINOLONE

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Introduction: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare and highly aggressive disease accounting for less than 1% of all cutaneous lymphomas. Unfortunately, there are currently no consensus guidelines for the treatment of this condition. Here we present the first known case of BPDCN in an older male treated with intralesional triamcinolone.

Report: A 61-year-old male with history of chronic thrombocytopenia presented to dermatology clinic with an asymptomatic, ecchymosis-like eruption on his chest with deep violaceous firm nodules on the face and back. He underwent imaging of his spleen which was unremarkable. Laboratory serology results for HIV, HCV, and H. pylori were negative. Three biopsies of his lesions that had clinically distinct morphologies were obtained. Histology showed small to medium sized cells with irregular contours, finely dispersed chromatin, and scant cytoplasm, staining positively for CD4, CD123 and TCL-1. Together, these findings were consistent with diagnosis of BPDCN. We referred him to oncology for further workup and treatment. The patient underwent a bone marrow biopsy that was suggestive of chronic myelomonocytic leukemia, and was started on palliative decitabine. At one-month follow up, his tumor nodules were significant enlarged and cosmetically bothersome. He was treated with intralesional triamcinolone on his forehead, left check and back. Three weeks later, there was marked improvement and near complete resolution of the treated nodules. Six weeks later, after the patient missed a cycle of decitabine, he had recurrence of his nodules. Further workup showed that he developed systemic mastocystosis consistent with overall disease progression. Oncology is working to transition him from decitabine to a second line chemotherapy drug.

Discussion: BPDCN typically presents in elderly patients, more often in men than women (3:1 male to female ratio) with a mean age between 60 and 70 years. Clinically, BPDCN typically presents with asymptomatic cutaneous lesions that progress to infiltrate the bone marrow. Mortality is high, with an estimated survival range of 9 to 20 months and an estimated five year survival rate is 0%. There are currently no consensus guidelines for treatment of BPDCN given its low incidence, clinically aggressive behavior and high mortality rate. Initial treatment typically consists of cyclophosphamide, doxorubicin, vincristine, and prednisone. Radiation for patients who are not candidates for chemotherapy has shown initial clinical regression followed by recurrence and progression of systemic disease. Bone marrow transplant has also been shown to slightly increase duration of remission before recurrence. In our patient, intralesional triamcinolone showed excellent initial tumor regression. Although his tumor nodules recurred, it is unclear whether this was a result of failing treatment or missing a cycle of his chemotherapy.

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

POSTER #95

GAMMA DELTA (ΓΔ) TYPE MYCOSIS FUNGOIDES IN AN OLDER ADULT MALE

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Introduction: Mycosis fungoides (MF) is a clonal T-cell lymphoproliferative disorder of the skin that accounts for an estimated 50-60% of CTCL cases. The gamma-delta (γδ) subtype of MF is the a rare variant, with only a few cases reported in the literature. Here, we present the case of an older male who developed gamma-delta type MF after liver transplantation.

Case: A 63 year old male presented to Dermatology clinic in February 2019 with red, scaly rash on the face and body present and worsening for over at least one year. He had a history of hepatitis C, alcohol induced cirrhosis, and hepatocellular carcinoma, status post liver transplantation in 2014, and was on dual-agent immunosuppression with everolimus and tacrolimus. The rash was itchy, tender and developed weeping, and he believed was spreading. Prior treatment with oral antibiotics, bacitracin, and benzoyl peroxide/clindamycin was ineffective. On exam, he was found to have a large erythematous plaque with erosion draining serous yellow fluid on the left mandible and neck, as well are multiple 1-5 cm well-defined pink to violaceous plaques on the chest, back, abdomen, buttocks, and bilateral upper and lower extremities. The patient was started on an 18-day prednisone taper, and biopsies of two lesions were obtained from his left lower cutaneous lip and left dorsal forearm. Both showed a patchy band-like atypical lymphoid infiltrate with epidermotropism, and stained positive for CD3, CD8 and gamma-delta T cell receptor, consistent with gamma/delta subset of CD8+ disease. PET/CT and peripheral blood flow cytometry revealed no obvious evidence of visceral disease and less than 5% burden in the peripheral blood. At one-month follow up, he had progression of disease with thickened skin lesions. After discussion with hematology/oncology, patient was started on bexarotene, and was given an additional tapering course of prednisone and instructed to take dilute bleach baths.

Discussion: MF typically has an indolent growth phase with an estimated 88% five-year survival rate. There are multiple subtypes of MF. The gamma-delta subtype is one of the rarest subtypes of MF and is characterized by the expression of the gamma-delta T-cell receptor. It can be difficult to distinguish this condition from primary gamma-delta T-cell lymphoma, as they can share clinical and histological patterns. Due to its rarity, there is limited data to guide therapy on gamma-delta type MF; there is no known cure, and treatment is primarily palliative.

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

POSTER #96

SYSTEMATIC REVIEW OF METASTATIC GLOMUS TUMORS: IMMUNOHISTOCHEMICAL CHARACTERISTICS AND OUTCOMES

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Background: Glomus tumors are rare, predominantly benign mesenchymal neoplasms that are derived from cells arising from within the glomus body, a thermoregulatory unit. Glomus tumors generally form from a neuroarterial structure composed of a thickened layer of modified smooth muscle cells that surrounds an extensive network of arteriovenous anastomoses and neural components. To date, there has been no comprehensive literature review of metastatic glomus tumors. Consensus guidelines do not exist for the diagnosis and surgical management of metastatic glomus tumors.

Objective: To compile all data on the characteristics and outcomes of metastatic glomus tumors.

Methods: A systematic review of PubMed, Web of Science, Cochrane, Excerpta Medica database (EMBASE) was performed to identify all cases of metastatic glomus tumors.

Results: Of the 809 abstracts from the literature search, 26 manuscripts with 35 metastatic glomus tumors met inclusion criteria. Location of primary tumor was commonly soft tissue (45.7%, 16/35), lung 22.9% (8/35), and stomach 17.1% (6/35). 39.3% (11/28) reported metastasis at initial presentation. IHC results showed 100% positivity for SMA (22/22), Vimentin (13/13), and p53 (3/3), and 91.7% (11/12) for Collagen IV. Negativity was 100% for S100 (16/16) and CAM 5.2 (5/5); 94.4% for Cytokeratin (17/18), and 93.3% (14/15) for CD34. Resection of the primary tumor was performed in 88.2% (30/34) of cases.

Lung was the most common site for metastasis (42.9%, 15/35), followed by brain (25.7%, 9/35), soft tissue (20.0%, 7/35), liver (17.1%, 6/35), intestine (17.1%, 6/35), lymph nodes (17.1%, 6/35), and bone (14.3%, 5/35). Other less common sites included the spleen, heart, adrenal glands, mesentery, kidney, peritoneum, thyroid and stomach. The average follow-up was 54.1 months. Rates of local recurrence (LRR) and mortality (MR) were 32.1% (9/28) and 63.3% (19/30), respectively.

Conclusions: Metastatic glomus tumors have high LRR and MR. Soft tissue was the most common primary site and lungs were most common site of metastasis. Immunohistochemistry findings showed high rates of positive staining for SMA, vimentin, p53, collagen IV and low rates of positive staining for desmin, S100, CAM 5.2, cytokeratin, and CD34. The IHC profile may aid in the diagnosis of glomus tumors with metastatic potential with markers of high sensitivity in diagnosis. More complete data are necessary to develop consensus guidelines for diagnosis and management of metastatic glomus tumors.

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

POSTER #97

MALIGNANT GLOMUS TUMOR OF THE BUTTOCK WITH PULMONARY METASTASIS

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Glomus tumors are rare, predominantly benign mesenchymal neoplasms that are derived from cells arising from within the glomus body, a thermoregulatory unit. Although glomus tumors most commonly arise in regions of increased glomus body concentrations, they are on occasion found in visceral locations such as the respiratory, gastrointestinal, and genitourinary tract. Glomus tumors are generally benign in nature. Here, we describe a rare case of a histologically-proven metastatic glomus tumor.

A 68-year-old female, who immigrated from a country in southeastern Africa, presented to an outpatient clinic with pain localized to the right buttock with swelling over five months. After one month of treatment with antibiotics for a presumed bacterial infection, the patient presented to a primary care facility to establish care. A review of her prior medical records revealed completion of eight cycles of an unknown chemotherapy regimen three years prior for an unspecified lung cancer in her home country within Africa, with no pre-treatment biopsies obtained. Complete blood count was measured and unremarkable at that time, and a comprehensive metabolic panel showed mild hypokalemia, mild hyperglycemia, and a mild elevation in aspartate aminotransferase. Lactate dehydrogenase was elevated to 459 units per titer with unremarkable PT/INR limits. Ultrasound of the right buttock to evaluate abscess formation showed a heterogeneous lesion within the right buttocks with internal blood flow that measured 8.7 cm x 5.9 cm x 9.6 cm. The lesion was identified as suspicious for malignancy and further chacterized with follow-up MRI, which showed a 10.9 cm x 9.8 cm x 7.2 cm diffusely enhancing, multi-lobulated mass with scattered cystic spaces that involved the gluteus maximus, extending into adjacent subcutaneous tissue. This was noted as concerning for soft tissue sarcoma. Biopsy was performed, resulting in lesional cells positive for SMA and negative for CKAE1/3 and S100, resulting in a diagnosis of glomus tumor. Due to her previous lung cancer, a CT of the chest was perfromed, showing eight distinct masses in the lungs bilaterally, with the largest in the right middle lobe measuring 4.7 cm x 3.5 cm in the axial plane. Additionally, a splenic mass and small pericardial effusion were noted. These findings were concerning for metastatic disease.

CT-guided biopsy of the right lung mass was performed. During the procedure, there was note of an increased vascularity of the pulmonary mass, with development of a small hemopneumothorax. Subsequent repeat CT scan with intravenous contrast showed stable pulmonary masses and a stable small right hemopneumothorax. The pericardial effusion was re-observed; however, due to its small size, no intervention was pursued. The patient was discharged on hospital day two with no adverse events.

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

POSTER #98

EXPLOITING TUMOR POSITION DIFFERENCES BETWEEN DEEP INSPIRATION AND EXPIRATION IN LUNG STEREOTACTIC BODY RADIATION THERAPY PLANNING

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Purpose/Objective(s): We introduce a novel stereotactic body radiation therapy (SBRT) planning technique that takes advantage of tumor motion relative to normal structures between respiratory states. We hypothesize that delivery of half a SBRT fraction dose in deep-inspiration breath hold (DIBH) position and the other half in the expiratory position spares more normal tissue compared to DIBH and expiration-alone plans.

Materials/Methods: Eligible patients had non-small cell lung carcinoma and previous lung SBRT in five fractions with available DIBH and expiratory computed tomography (CT) scans. Utilizing volumetric modulated arc therapy (VMAT), three different radiation plans were generated—one using a DIBH scan, another using an expiratory scan, and a third using a free breathing scan with corresponding target and adjacent chest wall contour. A fourth composite plan was generated delivering half the dose using the DIBH plan and the other half using the expiratory phase plan for each fraction. To generate the composite plan for accurate dose volume histogram (DVH) evaluation, the two scans underlying the two plans were fused based on ribs adjacent to the tumor.

Results: Five eligible patients had lesions located close to the chest wall. The median longest tumor diameter was 2.5cm (range: 0.7-2.9cm, n=5). The median superior-inferior tumor movement was 2.05cm (range: 1.4-2.9cm, n=4), and the measured anterior-posterior tumor movement for one patient was 2.9cm. The median reduction in the chest wall V30Gy for the composite plan was 98.3% (range 33.7-100%, absolute reduction: 6.3cc (range: 2.1-17.3cc)) compared to the inspiration phase alone plan and 98.2% (range 32.3%-100%, absolute reduction: 5.9cc (range: 3.2-16.6cc)) compared to the expiration phase alone plan, and 99.1% (range 69.7%-100%, absolute reduction: 25.8cc (range: 5.0-32.7cc)) compared to the free breathing plan. The median reduction in chest wall maximum dose for the composite plan was 32.9% (range 0.270%-46.4%, absolute reduction: 1424cGy (range: 15-2466cGy)) compared to the inspiration phase alone plan and 35.1% (range 3.18%-48.2%, absolute reduction: 1571cGy (range: 182-2650cGy)) compared to the expiration phase alone plan. The composite plan had a greater reduction in chest wall maximum dose if there was no overlap in planning target volumes (PTVs) between the inspiration and expiration target location relative to adjacent ribs (no overlap vs overlap, median 43.1% vs 5.21% reduction). Lung V20Gy were similar within 2.1% between different plans. The new composite DIBH-expiration plan theoretically allows for three of the five patients to receive three fraction SBRT to 54Gy instead of five fraction SBRT to 50Gy.

Conclusion: We conclude that composite deep inspiration-expiration SBRT planning has the potential to significantly improve organ at risk (OAR) sparing if there is sufficient tumor movement. This offers opportunities to lower toxicity risk, dose escalation, and for some, treatment in three fractions instead of five.

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

POSTER #99

HOME-BASED EXERCISE INTERVENTION IS FEASIBLE FOR PEDIATRIC CANCER PATIENTS

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Intro: Exposure to Vincristine in the first four weeks of pediatric cancer treatment has been shown to produce clinically significant neuropathy in greater than 75% of pediatric cancer patients. There are currently no effective treatments or interventions for vincristine-induced peripheral neuropathy (VIPN). This is a significant problem as the increase in metabolic and chronic health issues seen long-term in these survivors is currently hypothesized to be a result of the motor impairment incurred from VIPN. An efficacious intervention is urgently needed to address the long-term health issues these survivors currently face after developing VIPN.

The aim of this study is to assess the feasibility and acceptability of implementing a combined aerobic and resistance training exercise program as a therapeutic intervention to aid the pediatric cancer population in reducing the long-term effects of VIPN.

Methods: In order to begin assessing the feasibility of this exercise intervention we will recruit and enroll newly diagnosed ALL patients at Riley Hospital for Children who meet the inclusion criteria. Inclusion criteria for this study includes:(1) age = 5 and = 18 years, (2) have been diagnosed with acute lymphoblastic leukemia(ALL),(3) will undergo the standard of care treatment for ALL with vincristine, (4) will have a Total Neuropathy Score of 3 or greater at week 6 of treatment.

The exercise intervention will include both in-person exercise instruction sessions with a specialist and at-home video exercise sessions. These will occur over the course of 8 weeks after consent, enrollment, and initial functional assessment are complete. As patients complete their exercise sessions, heart rate, and activity will be monitored using a Fitbit and logged into the Fitabase database. iPads preloaded with 6 different videos detailing at-home exercise session options will be provided to guide the at-home sessions. Completion of at-home exercise sessions will be monitored using Fitabase in order to determine adherence.

Results: At this time, we have enrolled and consented 7 pediatric cancer patients in this study. All 7 completed initial assessments, have begun their exercise programs, and have successfully recorded data. Two of these 7 have fully completed the exercise program. Adherence in patients who have begun exercising is currently above 50% throughout. There have been no adverse events. Patient and parent satisfaction scores from participants having completed the intervention indicate they would recommend this program to others.

Conclusion: Based on current results and expectations, this 8-week exercise program is likely a feasible, acceptable, and safe intervention for the pediatric cancer population experiencing VIPN. Future robust randomized controlled trials are needed to confirm this finding.

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

POSTER #100

REDUCED CARDIAC AND PULMONARY DOSE WITH LEFT SIDED BREAST CANCER PATIENTS WHO RECEIVE ADJUVANT RADIATION THERAPY

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Purpose: To determine whether a 20-second breath hold during breast radiation reduces the amount of radiation to the heart and lungs.

Methods:

A retrospective chart review was done on 59 patients with left-sided breast cancer at Indiana University Ball Memorial Hospital from 2016-2017. To evaluate the impact that breath hold techniques have in terms of cardiac sparing, cardiac doses were evaluated for 29 consecutive patients prior to and 30 consecutive patients after the introduction of a deep inspiration breath hold technique. Patients were coached to hold their breath for 20 seconds during planning CT and radiotherapy, for a total of six to eight times during their radiation treatment. The control and the experimental group were compared on mean heart dose (Gray), maximum heart dose (Gray), volume of heart receiving 5 Gray (cc), and volume of left lung receiving 20 Gray (cc).

Results:

Mean heart dose was significantly reduced in those using the breath hold technique, averaging 1.481Gy (Std. Dev. 0.6511) in the control group, and 0.7653Gy (Std. Dev. 0.9046) with breath hold ($p<0.0001$). Average maximum heart dose was also significantly reduced with breath hold; 14.46Gy (Std. Dev. 11.31) compared to 31.88Gy (Std. Dev. 13.19) in the control group ($p<0.0001$). Volume of the heart receiving 5Gy of radiation was significantly reduced with breath hold ($p<0.0001$). Volume of left lung receiving 20Gy was also significantly reduced in the breath hold group($p<0.008$).

Conclusion: The results demonstrated a decrease in the cardiac dose with the use of a deep inspiration breath hold technique in women with left sided breast cancer who received adjuvant radiation therapy, which supports its continued use.

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

POSTER #101

MODELING LYMPHOCYTE LOSS KINETICS IN PATIENTS TREATED WITH FRACTIONATED RADIATION THERAPY

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Purpose: Radiation (RT)-induced lymphopenia (RIL) is associated with worse survival in patients with solid tumors as well as lower response rates to checkpoint inhibitors. This analysis aimed to model the kinetics of lymphocyte depletion during RT to assist in predicting RIL risk.

Methods: This registry-based study included 705 patients who received either total body irradiation (TBI; n = 30), stereotactic body RT (SBRT; n = 73), or conventionally fractionated chemoradiation (CFRT; n = 602). The CFRT group included patients with abdominal (n = 195), pelvic (n = 121), lung (n = 113), head and neck (n = 85), and central nervous system (CNS; n = 88) malignancies. For each patient, serial absolute lymphocyte counts (ALCs) were plotted against RT fraction number. The initial 3 weeks of treatment for CFRT patients and the entirety of treatment for SBRT and TBI patients were fit to exponential decay in the form $ALC(x) = ae^{-bx}$. From those fits, percent per fraction lymphocyte loss (FLL) was calculated as $FLL = (1 - e^{-b})*100$, and multivariable linear regression was performed to find its significant predictors.

Results: Curves were well fitted by exponential decay for all groups except for CNS patients (median linearized R^2 0.98, 0.93, 0.96, 0.67 for patients treated with TBI, SBRT, CFRT excluding CNS patients, and CNS patients respectively). In well-fit CFRT patients, apparent ALC loss rate slowed after week 3, potentially due to lymphocyte repopulation or other factors. TBI and SBRT patients completed RT before the end of the exponential decay phase, and their ALC loss rates remained unchanged throughout RT.

Median initial FLL varied significantly with treatment technique at 35.5%, 24.3%, and 9.4% for patients treated with TBI, SBRT, and CFRT, respectively ($p < 0.001$). Within the CFRT cohort, patients with abdominal (10.9%) and pelvic malignancies (10.1%) had higher FLL than those with lung (7.9%) or head and neck (7.5%) malignancies ($p < 0.001$). Significant predictors of FLL varied by site and included field size, dose per fraction, mean spleen dose, chemotherapy backbone, and age. In pancreatic cancer patients, gemcitabine was associated with a higher FLL (mean = 10.7) than 5-FU (mean = 8.3) after adjustment for covariates ($p < 0.001$). Finally, FLL was highly correlated with total % ALC loss during RT ($p < 0.001$) in all groups.

Conclusions: Lymphocyte depletion kinetics during the initial phase of fractionated RT are characterized by pure exponential decay. Initial FLL is strongly correlated with RT planning parameters and predicts total % ALC loss. The highest ALC loss rates were associated with RT-only regimens, implying that concurrent chemotherapy is not solely responsible for lymphopenia in patients receiving CRT. This work may also assist in selecting patients for adaptive RT approaches to mitigate RIL risk.

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

POSTER #102

PROGNOSTIC VARIABLES ASSOCIATED WITH IMPROVED OUTCOMES IN STAGE III NSCLC PATIENTS TREATED WITH CONSOLIDATION PEMBROLIZUMAB

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Background

HCRN LUN 14-179 is a phase II trial of consolidation pembrolizumab following concurrent chemoradiation for the treatment of patients with stage III NSCLC. Time to metastatic disease, PFS, and OS appear superior to historical controls of chemoradiation alone. Unfortunately, not all patients benefit from consolidation immunotherapy. We performed a univariate analysis evaluating variables associated with PFS, metastatic disease, and OS.

Methods

We conducted a retrospective analysis from patients enrolled on HCRN LUN14-179. Data collected included age, sex, stage, smoking status, PD-L1 status, >G2 vs <G1 adverse event, <G2 vs. >G3 pneumonitis, duration of pembrolizumab (<4 vs. >4 cycles), chemotherapy regimen, PS 0 vs 1, time to start pembrolizumab (<6 vs. >6 weeks from radiation), V_{20} (<20% vs. >20%), and total radiation dose. Univariate Cox regression was performed to determine the variables associated with 3 endpoints: time to metastatic disease/death; progression free survival; and overall survival.

Results

From April 2015 to December 2016, 93 patients were enrolled and 92 were included in the efficacy analysis (1 patient was ineligible). For time to metastatic disease or death, improved outcomes may be associated ($p<0.1$) with stage IIIA, non-squamous cell, >4 cycles of pembrolizumab, and $V_{20}< 20\%$. For PFS, improved outcomes ($p<0.1$) may be seen for females, stage IIIA, non-squamous histology, PD-L1 [-] tumors, >4 cycles of pembrolizumab, and $V_{20}< 20\%$. For OS, improved outcomes ($p<0.1$) may be seen for non-squamous histology, PD-L1 [-], >4 cycles of pembrolizumab, $V_{20}< 20\%$, and <G2 pneumonitis.

Conclusion

Non-squamous NSCLC, PD-L1 [-] tumors, and V_{20} <20% may be associated with prolonged time to metastatic disease or death, PFS, and OS for patients with stage III NSCLC treated with chemoradiation followed by pembrolizumab.

Translational/Clinical Research ***Medical resident***

POSTER #103

DURATION AND PATTERNS OF NEOADJUVANT THERAPY AND OUTCOMES OF PATIENTS WITH RESECTABLE AND BORDERLINE RESECTABLE PANCREATIC DUCTAL ADENOCARCINOMA (PDAC)

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Background: Neoadjuvant Therapy (NAT) may better select patients with PDAC for surgical resection. NAT could improve margin negative resection, allow systemic therapy to treat micrometastases early in the treatment course, and select out patients with aggressive disease who may not benefit from surgical resection. The role of radiotherapy (XRT) may be especially beneficial in borderline resectable (BR) PDAC, but its role has been debated with the use of multiagent modern chemotherapy. We compared survival outcomes of patients with resectable (R) and BR PDAC in varying durations of NAT with or without XRT.

Patients and Methods: Patients with R or BR PDAC who underwent NAT with or without XRT followed by curative resection were included. Data was extracted from an IRB approved protocol of a pancreatic cancer database at Indiana University. Overall survival (OS) was calculated from date of tissue diagnosis and compared between those receiving <3 months vs. =3 months of NAT and those receiving NAT with vs. without XRT. Treatment was administered based on the investigator's discretion and cases were discussed in a multi-disciplinary pancreatic cancer tumor board.

Results: Between summer 2008 and summer 2018, 116 patients received NAT with or without XRT and completed surgical resection. Median age was 63 years. Stages were R=43%, BR=53%. Of those patients with resectable disease and BR disease, 55% and 45% received <3 months NAT respectively. Most received modified FOLFIRINOX or FOLFIRINOX (59%) or gemcitabine/nab-paclitaxel (13%), and 24% received XRT. There were four complete responses, all in the =3 mo NAT with XRT. Percent node positive was lower in the NAT with XRT compared to NAT only (median 0% vs. 7.4%, $p<0.01$), but this did not differ by duration of NAT. At resection, a >50% necrosis was seen in 8% of those receiving =3 months of NAT compared to 1% of those receiving <3 months. Pathologic progression was seen in 4% of those with =3 months NAT vs. 15% in those with <3 months. With a median follow up time of 19 mo, there was no difference in OS in patients receiving <3mo NAT vs. =3 mo NACT (25.9 mo vs. 23.1 mo), though median OS in NAT with XRT was 26.5 mo vs. 24.3 mo in those receiving NAT without XRT ($p=0.01$). There was no difference in DFS in those receiving longer NAT or XRT.

Conclusion: In this study, patients who received NAT with XRT had improved mOS compared to those who did not receive XRT, while mOS did not differ by duration of NAT. Further analysis is being completed using propensity scoring to adjust for potential treatment selection bias and the role of postoperative adjuvant therapy.

Translational/Clinical Research ***Medical resident***

POSTER #104

ACTIVATED PLASMACYTOID DENDRITIC CELLS AS BIOMARKER AND INDUCER OF GRAFT-VERSUS-HOST DISEASE, RESPONSIVE TO ICOSL/VARIANT IGD INHIBITION

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Gastrointestinal graft-versus-host disease (GI-GVHD) is a leading cause of death post-allogeneic hematopoietic cell transplantation (HCT). Using proteomics, we identified and validated an increased $CD4^+CD146^+$ T cell population in GI-GVHD patients. Coexpression of interferon (IFN)g (Th1) and IL-17 (Th17) by this population was induced by Inducible COStimulator (ICOS), critical for Th17 (Li et al, *JCI Insights*, 2016). Thus, we next analyzed ICOSL expression on blood HLA-DR^+CD11c^+conventional (c) dendritic cells (DCs), and HLA-DR^+CD123^+plasmacytoid DCs (p)DCs in the same cohort of patients. The frequency of ICOSL was significantly higher on pDCs in GI-GVHD patients (n=64) compared to patients without GI-GVHD (n=39) or non-GVHD enteritis (n=22) (28%, 5% and 7%, all $p< 0.0001$).

We hypothesized that ICOS/ICOSL pathway blockade will prevent GI-GVHD development via decreased pDC and Th17 activation. The growth factor fms-related-tyrosine-kinase-3-ligand (Flt3l) promotes the development of pDCs, and Stat3, is required for Flt3l-dependent DCs differentiation. We first found that knocking out (KO) ICOSL in the donor bone marrow (BM) extended survival compared to wild-type (WT) mice in the major mismatch (B6H-2^b¿BALB/cH-2^d) HCT model while recipients of $Stat3^{KO}$ BM did not show difference in GVHD mortality. We next analyzed the GI immune cells at day 10 post-HCT, and found significantly lower frequencies of pDCs ($CD11b^-CD11c^+B220^+CD103^+$), and Th1/Th17 in recipients of $ICOSL^{KO}$ BM compared to recipients of WT BM. We also found a significant decrease of Flt3l levels in plasma collected at day 3 from $ICOSL^{KO}$ BM recipients compared to WT mice. For translational purpose, we used then the xenogeneic model with human PBMCs¿NSG and the ALPN-101 ICOSL vIgD-Fc (a human ICOS/CD28 dual antagonist). We tested a prophylactic regimen from Day-1 to Day+14 with 100 µg of ALPN-101, every other day, and showed significantly improved survival compared to the isotype control. Analysis of the GI showed a reduction in the infiltration of immune cells (0.24% vs. 2.8%, $p< 0.014$), the frequencies of DCs (0.42% vs 6.11 %, $p<0.007$) and $CD146^+$T cells (0 % vs.17.21%, $p< 0.017$) in the ALPN-101 group as compared to the control. Importantly, a therapeutic regimen from Day+7 (at the signs of GVHD) to Day+21 also improved survival, reduced infiltration of immune cells (0.056 vs 2.6%, $p< 0.0001$), frequencies of DCs (0% vs 3.5%, $p<0.007$) and $CD146^+$T cells (0% vs 12.3%, $p<0.0053$) in the ALPN-101 group compared to the control.

We conclude that $ICOSL^+$ pDCs represent a GI-GVHD biomarker. Targeting ICOSL may prevent and treat GI-GVHD.

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

POSTER #105

HIGHER SIROLIMUS LEVELS BETWEEN DAYS 11-20 AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HCT) INCREASE THE RISK OF HEPATIC SINUSUOIDAL OBSTRUCTION SYNDROME (SOS) AFTER NON-BUSULFAN MYELOABLATIVE CONDITIONING

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Background: Hepatic SOS is a potentially life-threatening complication occurring in up to 14% following HCT. Sirolimus(Siro) plus tacrolimus (FK) is an accepted regimen for graft-versus-host disease (GvHD) prophylaxis. We previously reported that higher trough Siro levels might increase the risk of SOS, although a small sample size precluded detailed analysis (*Pharmacotherapy* 2012; 32:441-5). In this study, we investigated if Siro and FK levels at specified times post-transplant are associated with an increased risk of SOS following non-busulfan, myeloablative conditioning.

Methods: We analyzed 260 consecutive patients with hematological malignancies treated at Indiana University from 2007 to 2016 who underwent allogeneic HCT following myeloablative conditioning using TBI-based (n=151) or non-busulfan chemotherapy only (n=109) regimens. Patients received Siro plus FK for GvHD prophylaxis. No prophylaxis for SOS was used. Trough levels of Siro (target 5-15 ng/mL) and FK (target 5-10 ng/mL) were obtained at least twice weekly through day 30.

Results: SOS occurred in 28 patients at a median of 22 (range, 12-58) days for a cumulative incidence of 10.8%. Nine patients died of other causes before day 40 and were excluded from further analysis. Siro and FK troughs were compared in patients who developed SOS (n=28) with those who did not (n=223). Baseline characteristics were similar between the two groups.

Mean Siro trough levels were higher between days 11-20 following transplant in patients who developed SOS (10.3 vs 8.5 ng/mL, P=0.008), while there was no significant difference in mean trough levels between days 0-10 (P=0.67) and days 21-30 (P=0.37) (Fig. 1). Patients with mean Siro trough level >9 ng/mL had higher incidence of SOS compared with those with Siro = 9 ng/ml (71.4% vs. 41.7%; P=0.003). No differences in mean FK levels during the same time intervals were observed between those developing SOS and others. On multivariable analysis, mean Siro trough level >9 ng/ml between days 11-20 post-transplant was associated with an increased risk of SOS (odds ratio [OR] 3.9, 95% CI: 1.6-9.8, P=0.003), together with a longer time from diagnosis to transplant (P=0.004) and use of a TBI-based regimen (P=0.006). Among patients who did not develop SOS, mean Siro trough levels =9 ng/ml between days 11-20 were not associated with an increased risk of grade II-IV acute GvHD at day 100 compared with levels >9 ng/mL(13.8% vs. 17.3%, P=0.99)

Conclusion: We conclude that mean trough Siro levels >9 ng/mL between days 11-20 post-transplant significantly increase the risk of SOS. Targeting lower Siro trough levels (e.g., 5-10 ng/mL) may reduce the risk of SOS without increasing acute GvHD. By interfering with endothelial cell function and potential healing following damage caused by TBI and/or high-dose chemotherapy, higher Siro levels could lead to SOS.

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

POSTER #106

OUTCOMES OF INFLIXIMAB IN MANAGEMENT OF STEROID-REFRACTORY ACUTE GRAFT VERSUS HOST DISEASE

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Background: Corticosteroids are considered the standard first-line treatment for moderate/severe acute graft versus host disease (aGVHD), yet there is no consensus on the optimal management of patients that are steroidrefractory (SR). Infliximab is a chimeric monoclonal antibody with anti-TNF-a activity that has previously demonstrated efficacy in SR-aGVHD.

Methods: We performed a retrospective review of 59 patients at Indiana University who underwent allogeneic stem cell transplantation and developed SR grade III-IV aGVHD from January 2007 to August 2018. Infliximab was administered 10 mg/kg weekly intravenously. Response criteria was in accordance with Martin et al (*BBMT* 2009; 12:777-784).

Results: Median age of treated patients was 49.5 years (range, 34.5-60) and median time from transplant to diagnosis of aGVHD was 106 days (range, 42-185) Patients received a median of 12 days (range, 8-20) of systemic steroids prior to infliximab. At the start of infliximab, 52 (88%) patients were on non-steroidal immunosupressants and 39 (66%) on non-absorbable steroids concurrently with systemic steroids. Patients received a median of 3 doses of infliximab with 37 (62.7%) patients receiving <4 doses. At 28 days after first infliximab dose, overall response was seen in 16 (27.1%) patients, with 8 patients achieving a complete response and 8 with a very good partial response. From the start of infliximab, the median overall survival was 104 days with 76.3% (n=45) non-relapse mortality and 5% (n=3) relapse mortality. Bacterial infections were seen in 41 (66%) patients treated with infliximab with median time to onset of 13 days (range, 6-24), and viral infections were seen in 31 (50%) of patients with median time to onset of 16 days (range, 9-43).

There was no significant difference between responders (n=16) and non-responders (n=43) in baseline demographic, transplant-related characteristics, initial GVHD prophylaxis regimen, number of non-steroidal systemic immunosuppressants at time of aGVHD diagnosis, or use of non-absorbable steroids. There was no difference in time from diagnosis of aGVHD to start of infliximab (median 16 vs. 11 days, P=0.06). Responders were less likely to have liver aGVHD involvement vs. non-responders (18% vs. 53%, P=0.016) and had slower tapering of systemic steroids, both at median time to 50% steroid dose (18 vs. 12 days, P=0.02) and 25% steroid dose (36 vs. 24 days, P=0.01). Responders had lower incidence of bacterial infection (44% vs. 74%, P=0.04), but not viral infections (56% vs. 47%, P=0.57). Responders had a longer overall survival (median 20.3 vs. 2.8 months, P=0.01).

Conclusion: Our single center report of 59 patients with SR-aGVHD treated with infliximab is among the largest reported. The outcomes of response to SR-aGVHD are poor and there is an unmet need for better agents. Additional studies with greater patient numbers are needed to identify factors associated with improved response to infliximab.

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

POSTER #107

MECHANISTIC ROLE OF MIR-29 IN PANCREATIC DUCTAL ADENOCARCINOMA PROGRESSION

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive cancers with a 5-year survival rate of 8%. Lack of curative treatment for PDAC warrants better understanding of its mechanisms. Our previous work demonstrated that miR-29 is commonly downregulated in PDAC, while its restoration exhibits tumor-suppressive effects. To understand these mechanisms, first we performed siRNA mediated knockdown of several miR-29 promoter binding transcription factors, including MYC, which play a role in PDAC. Our results indicate MYC represses miR-29 expression, and MYC nuclear localization negatively correlates with miR-29 in pancreatic cancer cells (PCCs). Next, to identify global miR-29 targets associated with PDAC, we conducted RNAseq on two miR-29 overexpressing PCC lines. This identified 41 overlapping miR-29 targets downregulated in both datasets. Gene ontology and survival correlation analyses identified seven most prominent targets (LOXL2, MYBL2, TRIB2, HGK, NRAS, CD276 and CLDN1). Ectopic expression of miR-29 significantly altered protein levels of these genes, confirming a translational suppression mechanism mediated by miR-29. RNAi mediated silencing of these targets significantly reduced the migratory ability of the PCCs. Our top target LOXL2 is involved in regulation of EMT/migration and extracellular matrix (ECM) remodeling. Luciferase reporter assay verified LOXL2 as a direct miR-29 target in PDAC. Immunohistochemical analyses in clinical specimens from PDAC patients and KPC mice pancreatic tissues showed higher LOXL2 expression in regions of pancreatic intraepithelial neoplasia (PanIN) lesions as compared to non-cancerous areas. This increased LOXL2 level was accompanied by concomitant reduction in miR-29 expression. Thus, our current study provides insight into new miR-29 mediated regulatory pathways in PDAC and reveals the association of miR-29-LOXL2 axis in the disease progression. Together, our data suggest miR-29 to play a critical role in mechanisms of PDAC progression and could serve as a potential therapeutic target for the disease.

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

POSTER #108

CALORIC RESTRICTION POTENTIATES THE THERAPEUTIC BENEFIT OF ANDROGEN DEPRIVATION THERAPY AND ALTERS MACROPHAGE POLARIZATION WHEN COMBINED WITH PD-1 INHIBITION IN MURINE MODELS OF PROSTATE CANCER

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Background: Data from epidemiological studies have linked dietary intake to the development of prostate cancer (CaP). We have previously shown that dietary protein restriction inhibits tumor growth by modulating PI3K/mTOR signaling *in vivo*. Additionally we have shown that dietary methionine restriction influence macrophage polarization which enhances the ability of the immune system to fight cancer. In this study we hypothesize that caloric restriction is capable of restricting tumor growth and potentiating the antitumor effect of androgen deprivation therapy (ADT) and PD1 inhibition. **Methods:** We utilized two prostate cancer models for our *in vivo* studies; castrate resistant LuCaP 23.1 AI prostate cancer model and MYC-Driven prostate cancer model in C57BL/6 mice. Caloric restriction was carried out by exposing mice to alternating fasting either as a single intervention (in LuCaP 23.1 model) or in combination with ADT (enzalutamide or surgical castration) or PD-1 inhibition in Myc-CaP model. Tumor sizes and weights were blindly assessed during the study and upon study termination respectively. IHC staining for both Ki-67 and mTOR phosphorylation was done to assess the impact of fasting+/- ADT on tumor growth and mTOR signaling. Macrophage polarization/distribution in tumors was assessed using immunofluorescence. Blood was collected at the end of the study for future comprehensive analysis of PBMCS. **Results:** In both models, alternating fasting was associated with significant decrease in tumor weight at the end of study in comparison to control group (*$p<0.05$). In Myc-CaP model, tumor weight was significantly decreased in combined fasting and enzalutamide/castration group in comparison to the control condition (**$p<0.001$) but was not significantly better than either intervention alone. Similarly, fasting potentiated the therapeutic benefit of PD-1 inhibition yet it was not significant. Combined fasting and ADT was associated with significant reduction in Ki-67 nuclear expression (****$p<0.0001$), however it was not significantly less than either agent alone. Combined fasting and ADT was associated with significant decrease in P-mTOR expression in comparison to control mice (****$p<0.0001$). Although we did not observe significant change in tumor weight with combined fasting and PD-1 inhibition; fasting potentiated the influence of PD-1 on macrophage polarization. M2 macrophages were significantly less in combined treatment compared to either single intervention alone (**$p<0.01$). **Conclusions:** Caloric restriction can hinder prostate cancer growth and potentiate the therapeutic effect of ADT. Our results provide basis for the translational use of dietary modification both as preventive measure as well as a therapeutic intervention that can improve the benefit of current standard treatments.

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

POSTER #109

NON-CYTOTOXIC DEGRADATION OF COREPRESSOR DNMT1 ENGAGES HEPATOCYTE DIFFERENTIATION TO SUPPRESS LIVER CANCER GROWTH

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Hepatocellular carcinoma (HCC) is the second most lethal malignancy in the world. Proliferation of HCC cells, mediated by the oncogene MYC, occurs without terminal hepatocyte commitment and differentiation. Meanwhile, hepatocyte precursor growth also mediated by MYC, is physiologically regulated by terminal differentiation producing functional hepatocytes. We found that hepatocyte lineage TFs (e.g.,FOXA1/2) that are wild-type in HCC fail to engage hepatocyte terminal differentiation by unknown mechanisms. We hypothesized that these TFs cooperate with transcriptional repressor proteins (e.g., DNA methyltransferase 1, DNMT1) to inactivate expression of hepatocyte differentiation genes. Using studies of chromatin immunoprecipitation with sequencing, hepatocyte lineage TFs (e.g, GATA4, FOXA1) strongly bound to the regulatory sites of hepatocyte terminal differentiation TFs (e.g, HNF4A, CEBPD). However, hundreds of hepatocyte epithelial differentiation genes were epigenetically repressed in HCC. We investigated FOXA1/2 transcriptional inactivity using immunoprecipitation tandem mass spectroscopy. These factors heavily recruited transcriptional co-repressor enzymes e.g., DNMT1 at baseline cell conditions. We then genetically validated DNMT1 as candidate molecular target in HCC using FANA Antisense Oligonucleotides (FANA-ASOs) that degrade target mRNA using HCC PLC cells. Degradation of DNMT1 using DNMT1-FANA (5nM) increased p27 protein, the marker of terminal differentiation, suppressed cell proliferation producing differentiation-based morphological changes and increased expression of hepatocyte differentiation TFs (e.g, HNF4A and CEBPD) found suppressed in HCC. Importantly, non-cytotoxic disruption of DNMT1 (0.25-1μM) with decitabine (Dec) modified FOXA1 interactions from corepressor to coactivator molecules, leading to induction of hepatocyte terminal differentiation. Cytidine analogs such as Dec are highly metabolized in the liver by a rate limiting enzyme cytidine deaminase (CDA) that is upregulated in solid tumor tissues such as HCC. Thus, we inactivated CDA using a competitive inhibitor tetrahydrouridine (THU, 1μM), followed by low dose treatment with Dec (0.25-0.5μM). This combination treatment potently suppressed HCC cell proliferation, increased p27 protein, and activated hepatocyte differentiation genes to increase terminal differentiation without engaging apoptosis induction (p-53 independent therapy). We next used an obesity induced mouse model of HCC, generated by treatment of mice with one dose of the carcinogen 7,12-Dimethylbenz[a]anthracene (DBMA) followed by feeding the mice with a high fat diet. These mice developed highly aggressive tumors in the liver starting at 11 weeks with an increased mortality starting at 30 weeks. Thus, we initiated treatment of the mice at week 11 using low dose dec treatment (0.2mg/Kg) three times weekly for 19 weeks with/without THU (n=5) mice per group in order to terminate the experiment at week 30. Decitabine treatment substantially decreased visible HCC tumors ($p<0.008$) and tumor burden ($p<0.03$) in this in-vivo proof of principle studies. In summary, we demonstrate that degradation of DNMT1 by non-cytotoxic concentration of decitabine decreases HCC growth in-vivo by differentiation induction and is an alternative therapy to current ineffective apoptosis-based therapies.

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

POSTER #110

GENE CO-EXPRESSION NETWORKS RESTRUCTURED BY GENE FUSION IN ALVEOLAR RHABDOMYOSARCOMA

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Alveolar rhabdomyosarcoma (ARMS) is the most common pediatric soft tissue sarcoma that harbors a diverse range of genetic mutations. In a majority of cases, pediatric ARMS present with a recurrent chromosome translocation between FOXO1 and either PAX3 or PAX7. The fusion protein (FOXO1-PAX3/7) is a potent transcriptional regulator for oncogenesis and disease progression. Many studies have compared gene expression differences associated with this gene fusion in ARMS; however, the change of gene interactions with respect to the fusion has not been studied. In this work, we examined gene co-expression networks (GCN) in microarray data of ARMS with fusion negative and positive samples. 41 gene co-expression modules were identified with the weighted network mining tool lmQCM, of which 17 showed significant association to fusion status. The GO analysis revealed significant structural changes may account for the co-expression for some of these modules. 109 GCN modules were identified for fusion negative samples only, whereas only 53 were observed in fusion positive ones. Not much enriched biological processes were identified for the GCN modules, which indicates that the same as FOXO1 alone, FOXO1 fusion acts on global and diverse pathways. Surprisingly, different expression
were observed in PAX3 vs. PAX7 fusion samples, which are enriched with cell death pathway and cellular signaling pathway genes, and the importance will be further investigated.

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

POSTER #111

ELUCIDATING PHOSPHOLIPID-MEDIATED EPIGENETIC ALTERATIONS IN OVARIAN CANCER STEM CELLS

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Epithelial ovarian cancer (OC)isthedeadliestgynecologicalmalignancy.Recurrence of advanced, multidrug-resistant metastatic disease after initially good response to standard lines of chemotherapy occurs in the majority of OC patients. Chemoresistant, recurrent OC is essentially fatal. Disease relapse is due to survival and expansion of a small pool of chemorefractory ovarian cancer stem cells (OCSCs). OC evolution into a platinum-resistant state is closely associated with accumulated epigenetic alterations, including altered DNA methylation and histone modifications. In addition, intraperitoneally residing OC cells are exposed to a variety of external cues arising from the ascitic microenvironment, which potentially contribute to cell stemness and chemoresistance. Bioactive molecule lysophosphatidic acid (LPA) is prominently present in the intraperitoneal fluid of OC patients and implicated in ovarian carcinogenesis and metastasis through a variety of mechanisms. However, limited data exist regarding potential epigenetic regulation of OC cells by LPA. The link between LPA and OCSC acquisition/chemoresistance has not been investigated. In the current study, we tested the hypothesis that LPA epigenetically mediates OCSC promotion and survival. An Aldefluor assay was employed to evaluate the expression of aldehyde dehydrogenase (ALDH), an accepted functional marker of cancer stem cells, in a panel of high grade serous ovarian cancer (HGSOC) cell lines (OVSAHO, OVCAR3, OVCAR5, PEO1, and Kuramochi) We here demonstrate that treatment of OC cells with increasing (0- 80uM, 72h) doses of LPA enriches ALDH+ population in a cell line specific dose-dependent manner (with ALDH expression increase peak at 10uM LPA treatment in PEO1 and Kuramochi cells, and ALDH expression increase peak at 80uM LPA treatment in OVCAR3 and OVSAHO; $p<0.05$). In accordance with flow cytometry enrichment analysis, we observed enhanced ($p<0.05$) stem cell properties in LPA-exposed (0-80uM, every 72hr, up to 21 day) cells via *in vitro* clonogenic assay. Functional assessment of LPA-induced OCSCs via in vitro tumor sphere formation and platinum sensitivity/MTT assays is currently underway. Fluorescence-activated cell sorting of OCSCs and non-OCSCs after LPA treatment will be followed by global molecular assessment of epigenetically altered genes/pathways in LPA-exposed and non-exposed cell groups via RNA-sequencing, Methyl-Capture-sequencing and subsequent integrated transcriptome/methylome analysis. Examination of stemness-promoting pathways and evaluation of specific pathway components will reveal new therapeutic targets to abrogate OCSC enrichment (and possibly revert the existing OCSC into non-stem cell state) in patients with high intraperitoneal and/or plasma levels of LPA.

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

POSTER #112

AKT1-MEDIATED ACTIVATION OF HSF1 BY PHOSPHORYLATION AND AN ASSOCIATION WITH METASTASIS-FREE SURVIVAL

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Breast cancer is the most common cancer affecting women worldwide. The overwhelming majority of deaths from breast cancer are caused by metastasis. The transcription factor heat shock factor (HSF1), which classically regulates the heat shock response, has been linked to tumor progression and metastasis. Our previous data indicate HSF1 is activated in approximately 50% of patient breast tumors, independent of subtype and is associated with promoting epithelial-to-mesenchymal transition (EMT). Our laboratory has shown that AKT1 phosphorylates Ser326 of HSF1, leading to HSF1 activation in breast cancer cells and EMT. However, it remains unclear what phosphorylation sites are most critical for HSF1 transcriptional activity and whether AKT1 also regulates other HSF1 sites. To answer this question, HSF1 was subjected to an in vitro kinase assay. We observed Ser326 phosphorylation via immunoblotting. These phosphorylated HSF1 proteins were further subjected to mass spectrometry wherein we identified three novel sites of AKT1-mediated phosphorylation in Thr142, Ser230, and Thr527. All of these sites, along with Ser326, have previously been shown to promote HSF1 transcriptional activity. Interestingly, incubation with a pan-AKT inhibitor also suppressed the heat shock response by HSF1. However, when HSF1 protein was incubated with AKT2 or AKT3, there was no phosphorylation at Ser326. Furthermore, transcriptional activity of HSF1 and expression of Hsp70, a known HSF1 target gene, were increased by AKT1 but not by AKT2 or AKT3. Thus, we have further identified that AKT1 phosphorylates several activating residues on the HSF1 protein whereas AKT2 and AKT3 do not phosphorylate HSF1 to promote its activation. The importance of promoting HSF1 transcriptional activity by AKT1 cannot be understated as the PI3K pathway has been found to be genetically activated in ~77% of breast cancer. Additionally, it has recently been reported that this pathway is activated in almost 40% of all human tumors. However, whether HSF1 activity has any relation to EMT or metastasis in these other tumor types is unknown. To address this, we developed a gene expression signature for HSF1. We found this gene signature was strongly associated with metastasis-free survival in a broad range of solid tumors including breast cancer, lung cancer, ovarian cancer, melanoma, pancreatic cancer, and prostate cancer. In summary, we established a more definitive mechanism by which AKT1 phosphorylates and activates the HSF1 protein by phosphorylation of several activating sites. Furthermore, due to the seemingly ubiquitous nature of PI3K/AKT signaling across human tumors, we found that HSF1 is also potentially activated in these tumor types and has a strong association with patient outcomes, in particular metastasis.

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

POSTER #113

DO SKELETAL MUSCLE AND ADIPOSE TISSUE HAVE DISTINCT GENE EXPRESSION PATTERNS IN PDAC CACHEXIA?

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Background: The vast majority of patients with pancreatic ductal adenocarcinoma (PDAC) suffer cachexia, a debilitating condition that reduces performance status, tolerance to anti-cancer therapies eventually affecting the quality of life and reducing survival. Although weight loss in cachexia results from concurrent loss of adipose and muscle tissue, most studies focus on muscle. Emerging data demonstrate prognostic value of fat loss, as well as implicate adipose tissue loss in muscle wasting, suggesting that adipose is an important, active component of cachexia. Here, our objective is to identify the muscle and adipose genes and pathways regulated in cachexia and to validate our findings against available external datasets.

Methods: Matched rectus abdominis muscle and subcutaneous adipose tissue were obtained at surgery from patients with benign conditions (n=11) and patients with PDAC (n=23). Gene expression was measured by Ion proton sequencing using a panel of well-characterized probes. Differentially expressed (DE) between controls and cancer were identified. Self-reported weight loss and body composition measurements defined cachexia status. Ingenuity Pathway Analysis (IPA) were used for pathway analysis. Spearman's rank correlation test was used to correlate the DE genes from muscle and adipose to cancer weight loss (CWL).

Results: The number of genes in adipose is ~ 5 times more than muscle indicating dynamic changes in adipose and may potentially precede muscle wasting. Most of the genes were unique to adipose and muscle demonstrating a tissue specific gene expression pattern. Although there were 9 common signaling pathways common between adipose and muscle, the genes involved activating or inhibiting those pathways are predominantly different emphasizing that adipose and muscle wasting may be mediated through independent mechanisms. For example, the top pathway, EIF2 signaling, had 87 genes from adipose but only 27 from muscle, with 18 genes in common. In both ours and the external dataset, we identified many well characterized cachexia genes in muscle such as IL6R, ZIP14, FOXO1, PDK4, FOXO3 which can be viewed as a cornerstone to develop a core set of common genes between model system and humans in cachexia. Similar investigation of adipose would provide an opportunity to develop a more comprehensive list of genes involved in cachexia.

Conclusion: This is the first study to perform a matched muscle and adipose gene profiling from a single cancer type and validate the findings in external dataset. We observed distinct, tissue-specific gene expression profiles between tissues, providing potential therapeutic opportunities in targeting adipose wasting along with current preclinical and clinical trials that are in various phases for improving muscle wasting.

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

POSTER #114

CLINICAL AND PATHOLOGIC OUTCOMES FOLLOWING HOLMIUM LASER ENUCLEATION OF THE PROSTATE IN MEN UNDERGOING ACTIVE SURVEILLANCE FOR PROSTATE CANCER

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Introduction and Objective:

Patients with prostate cancer (PC) who wish to pursue active surveillance (AS) present a unique challenge to the urologist if these men have lower urinary tract symptoms (LUTS) or urinary retention. The objective of this study was to describe clinical and pathologic outcomes for patients on AS for PC who also underwent holmium laser enucleation of the prostate (HoLEP) for LUTS or urinary retention.

Methods:

Through an IRB-approved protocol, we prospectively collect preoperative, perioperative, and postoperative data on all patients undergoing HoLEP at our institution. We queried this database to include all men with a known diagnosis of PC who chose active surveillance as primary treatment for their malignancy. We excluded men who were planning to undergo radiation as primary cancer treatment following debulking HoLEP due to obstructive LUTS. Perioperative and postoperative patient characteristics were evaluated.

Results:

We included 71 patients for analysis (Table 1). The median patient age was 74 years, and 38% of patients required an indwelling catheter or intermittent catheterization for urinary retention before surgery. Preoperatively, the majority of patients had Gleason sum 6 PC and the median PSA for the group was 9.0 ng/mL (IQR 5.9-13.6). The median weight of tissue removed was 73 grams (IQR 37-101). Most patients (63.4%) had no malignancy on HoLEP pathology, and the three patients with preoperative Gleason 7 and 8 had either Gleason 6 or no cancer on HoLEP pathology. Over a median 12-month follow-up period, all patients were free of urinary retention, and 90.1% of patients remained on AS without plans for other therapy. Median AUA symptom scores were significantly lower postoperatively compared to preoperatively (6 vs 22, respectively; $p<0.01$). Additionally, the PSA decreased to a median of 1.4 ng/mL (IQR 0.7-3.5).

Conclusions:

In patients with LUTS or urinary retention wishing to undergo AS for PC, HoLEP will provide many men significant symptomatic improvement and relief of urinary retention while allowing them to continue this treatment option.

Table 1. Demographics, perioperative outcomes, and postoperative outcomes following HoLEP in patients undergoing active surveillance as primary treatment of prostate cancer.

Median Age in Years (IQR)	74 (69-78)

Indwelling Foley or CIC at Time of Surgery, n (%)	27 (38.0)
Preoperative Median (IQR) AUA Symptom Score	22 (17-26)
Preoperative Gleason Sum Score, n (%) Unknown 6 7 8 9 10	 30 (42.3) 38 (53.5) 2 (2.8) 1 (1.4) 0 0
Median Preoperative PSA (IQR) in ng/mL	9.0 (5.9-13.6)
Median Enucleation Time in Minutes (IQR)	59 (41-90)
Median Morcellation Time in Minutes (IQR)	13 (5-18)
Median Enucleated Specimen Weight in Grams (IQR)	73 (37-101)
Number of Patients with No Malignancy on Pathology (%)	45 (63.4)
Postoperative Gleason Sum Score, n (%), of Patients with Malignancy on HoLEP Pathology Unable to Score / Unknown 6 7 8 9 10	 12 (46.2) 12 (46.2) 1 (3.8) 0 1 (3.8) 0
Median Months of Follow-up (IQR)	12 (6-12)
Lowest Postoperative PSA in ng/mL	1.4 (0.7-3.5)
Repeat Resection of Prostate Tissue	0
Postoperative Urinary Retention, n (%)	0
Postoperative Median (IQR) AUA Symptom Score	6 (3-10)
Postoperative Primary Treatment Active Surveillance Primary Androgen Deprivation Primary Radiation Radical Prostatectomy	 64 (90.1) 4 (5.6) 2 (2.8) 1 (1.4)

POSTER #116

TUMOR SUBTYPE-SPECIFIC ABILITY OF MIR486 IN OVERCOMING MAMMARY TUMOR-INDUCED FUNCTIONAL LIMITATION

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Mir-486 expression is enriched in skeletal muscles and this microRNA is an integral part of the myogenesis signaling network that involves Pax7, MyoD, myostatin and NF-kB. Reduced mir-486 expression in muscle is a major defect in muscular dystrophy. Muscle-specific transgenic expression of mir-486 using muscle creatine kinase promoter (MCK-miR-486) is sufficient to rescue muscular dystrophy phenotypes in animal models. We had previously demonstrated reduced circulating and skeletal muscle miR-486 in mice bearing mammary tumors, which correlated reduced skeletal muscle activity and functional limitation. Circulating miR-486 was lower in breast cancer patients with metastatic disease compared to healthy women. Therefore, cancer-induced systemic effects could involve altered mir-486 levels in muscle, which creates a condition similar to "muscular dystrophy" in cancer patients. This study investigated the hypothesis that restoration of skeletal muscle miR-486 expression ameliorates mammary tumor-induced muscular dystrophy phenotype and functional limitations. We employed MMTV-PyMT and MMTV-Neu transgenic mice as animal models of human breast cancer to characterize tumor progression and associated functional limitations. These mice were crossed to MCK-miR-486 mice of the same genetic background to increase skeletal muscle miR-486 levels in tumor-bearing mice. Resulting females were characterized for tumor progression and functional limitations. We found that mammary tumor occurred as soon as 7 weeks in MMTV-PyMT mice and approximately 20 weeks in MMTV-Neu post birth dates along with reduced functional performance such as reduced grip strength, impaired rotarod balance and reduced muscle contraction force. Double transgenic MMTV-PyMT/mir-486 and MMTV-Neu/mir-486 female mice displayed significantly slower tumor occurrence along with differences in body compositions compared to MMTV-PyMT and MMTV-Neu mice, respectively. Furthermore, overexpression of miR-486 significantly prevented tumor-induced reduction in grip strength and rotarod performance and accumulation of free water in MMTV-Neu mice, but not in MMTV-PyMT mice. These data suggest that mir-486 alone can restore skeletal muscle function in specific subtypes of mammary tumors. Further studies are necessary to determine mechanistic aspects of tumor subtype specific effects of skeletal muscle miR-486 in reversing tumor-induced functional limitations.

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

POSTER #117

MICROSATELLITE INSTABILITY MAY PREDICT RESPONSE TO SIPULEUCEL-T IN PATIENTS WITH PROSTATE CANCER

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Background: Microsatellite instability (MSI) is a mutational error in repetitive DNA sequences that is typically corrected by a DNA mismatch repair system. A failure in this system leads to defective mismatch repair (dMMR). Recent data shows that 2.4% of prostate cancers are MSI-H and up to 8.1% show evidence of dMMR. Currently, pembrolizumab, an immunotherapy-based PDI-1 inhibitor, is approved for any MSI-H/dMMR cancer. MSI-H tumors may have higher tumor mutational burden and produce a number of neoantigens that trigger the recruitment of tumor-infiltrating lymphocytes. As a result of this, these MSI-H tumors strongly express immune checkpoints ligands, such as PD-1, to promote immune evasion.

Sipuleucel-T is an autologous immunotherapeutic vaccine approved in 2010 for the treatment of mCRPC that involves activating the patient's own dendritic cells. These are exposed to a fusion protein, PA2024, consisting of antigen prostatic acid phosphatase (PAP) and GM-CSF. After incubation, the blood product is reintroduced to the patient over three treatments, stimulating an immune response against the prostate cancer.

Method: A 71 year-old man with metastatic castration resistant prostate cancer presented initially in 2004 with Gleason 3+4 prostate adenocarcinoma. He underwent radical prostatectomy. In 2007, the patient was found to have biochemical recurrence of prostate cancer and underwent salvage radiation therapy. He then developed metastatic disease to the lung in 2012. A chest CT scan demonstrated a right lower lobe pulmonary nodule, found to be prostate adenocarcinoma on FNA. He was treated with androgen deprivation therapy with leuprolide for approximately one year, Following a subsequent demonstration of disease progression, patient received 3 cycles of sipuleucel-T starting 9/2014. He responded remarkably well with normalization of PSA and complete response of his disease by RECIST criteria. Whole genome sequencing was done on the patient's disease to explore potential future treatment options. Whole genome sequencing demonstrated high mutational burden in addition to microsatellite instability (MSI).

Discussion: There is limited information on dMMR prostate cancer and its response to sipuleucel-T immunotherapy. Patients with dMMR/MSI-H showed significant response to immune checkpoint inhibitors and the FDA granted approval of pembrolizumab in all cancer that are MSI-H. However, there is no data about the response to cell-based immunotherapy such as sipuleucel-T in patients with dMMR/MSI-H.

An interesting observation in this patient is his complete response to sipuleucel-T and whether this related to MSI-H status. To the best of our knowledge, this is the first case of a complete response to sipuleucel-T in a known dMMR metastatic castrate-resistant prostate cancer. Whole genome sequencing may play a role in selecting appropriate prostate cancer patients for sipuleucel-T. Ultimately, more research needs to be conducted in order to better understand the response of sipuleucel-T in MSI-H/dMMR prostate cancers.

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

POSTER #118

ROLE OF RHOX10 IN OVARIAN CANCER

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Ovarian cancer effects one in seventy-five women, ranking as the fifth leading cause of cancer associated deaths. An aggressive subtype, high-grade serous carcinoma (HGSOC) accounts for approximately 74% of ovarian cancer mortalities. Furthermore, a novelty group of cancer stem-like cells (CSC) are believed to induce malignant dissemination. Identifying initiation mechanism of CSCs can improve HGSOC prevention and early detection. Molecular and cellular parallels of clinical HGSOC have been developed via a Dicer-Pten double knockout (DKO) and Dicer-Pten-p53 triple knockout (TKO) mouse model. The disease model supports HGSOC primary origin in the fallopian tube instead of the ovaries. Rhox10, a gene preferentially expressed in the reproductive system, reports to play a role in cell migration and stem cell differentiation in adults. For this reason, Rhox10 is expected to promote metastatic growth or affect the differentiation of fallopian tube stromal cells into ovarian CSCs. RNA Seq results of primary tissue reveal an increased expression in a step-wise pattern as HGSOC progresses from early to advanced stage. Ovarian cancer cell lines and primary mouse tumor tissue were analyzed for validation by mRNA quantification and immunohistochemistry, respectively. Quantification of Rhox10 expression in DKO and TKO cell lines confirm an extensive upregulation of the gene relative to the normal fallopian tube. In addition, Rhox10 expression is hyperregulated in the CSC population. These observations suggest a correlation between Rhox10 expression and HGSOC progression via the spheroid mediated mechanism. In the future, stable RNA interference will render gene deficient effects on in-vitro CSCs spheroid formation and cellular characteristics to elucidate the importance of Rhox10 in ovarian cancer.

Translational/Clinical Research ***Post-baccalaureate fellow***

POSTER #119

SINGLE-CELL RNA EXPRESSION OF HUMAN IPSC-DERIVED NEURONS

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Taxane-induced peripheral neuropathy (TIPN) is the most clinically important side effect of paclitaxel and can be devastating and dose limiting. We have previously found that dose reductions due to TIPN in the curative setting lead to inferior outcomes for African American patients. The mechanism of TIPN, however, has not been entirely elucidated. To help understand the mechanistic underpinnings of TIPN, our laboratory has investigated paclitaxel-induced neuronal morphological and molecular changes using an *ex vivo* model of human-induced pluripotent stem cell (iPSC)-derived neurons (iPSC-iSNs). Neurite outgrowth measurement and single cell sequencing has been performed on mature iPSC-iSNs, both with and without paclitaxel treatment to assess the impact of treatment on morphology and to better characterize the ex vivo model. This work will allow for subsequent studies on enriched sensory neuron populations to evaluate epigenomic and transcriptomic changes in the iPSC-iSNs after treatment with paclitaxel.

We have performed a cell viability assay and fluorescence-activated cell sorting (FACS) using four cell-surface markers (CD184, CD44, CD15, and CD24) to select a neuronal population for sequencing. Within the isolated neuron population, more than 70% of the cells were viable and therefore available for single-cell sequencing. The single cell 3' RNA-sequencing experiment was conducted using the Chromium single cell system (10x Genomics, Inc) and the resulting library was sequenced with the NovaSeq 6000 sequencer (Illumina, Inc). The sequence data generated were first processed with CellRanger 3.0.2 (http://support.10xgenomics.com/) and the resulting gene-cell barcode matrices were further analyzed with Seurat 3. Clusters identified using Seurat 3 demonstrated the heterogeneity of our mature iPSC-iSN population. Preliminary automatic annotation of the cell clusters suggested that the majority of these clusters were characteristic of neurons, with smaller numbers of other cell types present, including astrocytes, fibroblasts, and neuroepithelial cells. Additional results from single-cell RNA sequencing may help to further characterize the subtypes of neurons generated from iPSCs and, in the future, may provide insight into the epigenetic changes that may occur in nerve cells as a result of paclitaxel treatment.

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

POSTER #120

PREDICTORS OF FAILURE AND SURVIVAL IN PATIENTS WITH HEPATOCELLULAR CARCINOMA TREATED WITH STEREOTACTIC BODY RADIATION THERAPY

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Purpose/Objective(s): Stereotactic body radiation therapy (SBRT) has been used as an effective treatment or as a bridge to liver transplant in patients with hepatocellular carcinoma (HCC). Despite high published rates of local control, failures can occur. In this study, we report our single institution experience and analyze treatment- and patient-related factors predicting for failure and overall survival.

Materials/Methods: We performed a retrospective analysis of HCC patients treated with SBRT at our institution between 6/2007 and 1/2017. Biological effective dose (BED) was calculated using alpha/beta = 10. Failure was defined as in-field if happened within 80% isodose line, out-field if failure happened outside 80% isodose line but within the liver, and distant if failure occurred outside the liver. Absolute lymphocyte count (ALC) nadir was defined as the lowest ALC within 2 months after SBRT and the cut off used to define low ALC nadir was < 0.5 k cells/ul. Statistical analysis was done using chi-squared testing, logistic regression, and Kaplan -Meier methods.

Results: We identified 92 patients with a median age of 63 years (IQR 57-76 years); 71% were male; 72% had Child Pugh A and 64% had ECOG of 1. The most common cause of HCC was hepatitis C (56%) followed by non-alcoholic steatohepatitis(24%). The median tumor size was 26 mm (range 12-148 mm), with a median dose of 48 Gy (range 20-50 Gy) and median BED of 124.8 (range of 28-133). With median follow-up of 19.5 months, there were 21 (22.8%) patients with any failure: 5 (22%) were in-field, 13 (%64) were out-of-field, and 5 (%22) were distant metastases. Of note, 2 patients had concurrent in- and out-field failure. Progression free survival was 18 months (range 2-97 months). The mortality rate was 36% and 52% in patients who developed any type of failure. Univariate analysis revealed that pre-treatment alpha fetoprotein (AFP) is associated with risk of any failure (p=0.04). In addition, pre-treatment International Normalized Ratio (INR) is associated with risk of in-field failure (p=0.007). BED or dose were not associated with any failure (p=0.49, p=0.37). Patient with low ALC nadir had a lower overall survival (OS, 13 months versus 30 months, p-value=0.006) Baseline, 3-month and 1-year AFP were not associated with time to any failure (p=NS). In-field failure was associated with improved OS (p=0.03). Distant failure was associated with higher rate of mortality (p=0.004).

Conclusion: We report our single institution experience of treating HCC patients with SBRT to define factors associated with failure and survival. The rate of any failure was 22.8% with low in-field failure (5.4%) after SBRT. The only factor associated with any failure was baseline AFP. Low ALC nadir was also associated with lower OS. These factors should be evaluated prospectively for validation.

Translational/Clinical Research ***Resident***

POSTER #122

CHARACTERIZATION OF CANCER CACHEXIA IN HEAD AND NECK CANCER PATIENTS UNDERGOING FREE FLAP RECONSTRUCTION

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Introduction: Cancer cachexia is the main cause of death in up to one third of cancer patients and affects 30% of patients with head and neck cancer at time of treatment. Data generated from experimental animal models and clinical studies demonstrate that cachexia generally associates with lean muscle wasting, chronic systemic inflammation and alterations in metabolism. Altogether, these derangements lead to poorer outcomes, worsened response to treatment, and overall reduced quality of life. The primary goal of this study is to characterize clinical, laboratory, and histologic features of cancer cachexia in patients with head and neck cancer undergoing surgery with free flap reconstruction. The hypothesis of this study is that patients affected with cancer cachexia exhibit morphologic and molecular changes (primarily consistent with systemic inflammation, muscle atrophy and muscle-specific modulation of gene expression) that uniquely distinguish them from patients without evidence of cancer cachexia.

Methods:A prospective study was conducted after obtaining regulatory approval of consenting adults with head and neck cancer who underwent head and neck surgery with reconstructive autologous free tissue transfer and had appropriate imaging. CT scans were reviewed at the third lumbar (L3) vertebra to calculate skeletal muscle index (SMI). Sarcopenia was defined as SMI = 41.6 cm^2/m^2 for males and = 32.0 cm^2/m^2 for females. Peri-operative laboratory data(C-reactive protein and albumin) and clinical factors were collected to a comprehensive analysis of factors that may contribute to cachexia. Tissue (e.g. muscle, bone) was collected in the operating room that would normally be discarded from the free flap during reconstruction and compare differences in histology and activation of cachexia-associated pathways.

Results: Preliminary data will be presented on subject stratification, demographics, SMI, modified Glasgow Prognostic Score, co-morbidities, and tissue analysis on the patient population to date (n=11+).

Conclusion: The results of this study will be the first in depth characterization of tissue in head and neck cancer patients with cachexia and can provide critical understanding of the mechanism of this disease.

Translational/Clinical Research ***Resident***

POSTER #123

COST ANALYSIS OF AUDIOVISUAL-ASSISTED THERAPEUTIC AMBIANCE IN RADIATION THERAPY (AVATAR) AIDED OMISSION OF ANESTHESIA IN RADIATION FOR PEDIATRIC MALIGNANCIES

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PURPOSE

Radiation therapy (RT) is a hallmark of optimal pediatric oncology care. Due to the inability of children to reliably remain immobile while receiving RT for cancer anatomy targeting requiring millimeter precision, daily anesthesia plays a large role. Pediatric RT involves a single-day simulation session followed by multiple consecutive daily treatment sessions ranging from 2-6 weeks in duration. Consequently, pediatric anesthesia for each RT session is a source of financial burden for patient families and the overall healthcare system in addition to being invasive, traumatic, and detrimental to patient quality-of-life. This study attempts to assess the cost-savings benefit of audiovisual-assisted therapeutic ambiance in radiation therapy (AVATAR)-aided omission of pediatric anesthesia in RT.

METHOD AND MATERIALS

The baseline time of anesthesia during RT was derived from documented anesthesia billing time during RT simulation at our institution and in published literature. Current procedural terminology and relative value unit codes encompassing anesthesia-related charges from Radiation Oncology and Anesthesia were analyzed in concert with this value to calculate the total cost of pediatric anesthesia per RT session. The number of RT sessions per patient was derived from the mean sessions of AVATAR-treated patients from the literature and our own institutional experience.

RESULTS

The mean number of RT fractions administered per patient with AVATAR-aided anesthesia omission at our institution was 19.5, similar to the 17.6 previously reported. At a mean anesthesia time exceeding 30 minutes (with mean RT duration of 4 weeks), the cost of pediatric anesthesia per RT fraction in non-AVATAR sessions was $1,950.68, yielding a total anesthesia cost of RT treatment of $38,233.24 per patient (including simulation). Patients at our institution were not billed for AVATAR-assisted RT.

CONCLUSION

The ability of AVATAR to obviate the necessity for daily anesthesia in pediatric RT provides substantial cost-savings. These findings argue for increased utilization of AVATAR and for analyses of RT targeting accuracy of AVATAR versus conventional anesthesia-guided treatment of pediatric malignancies.

Translational/Clinical Research ***Resident Physician***

POSTER #124

BASELINE KARNOFSKY PERFORMANCE STATUS IS INDEPENDENTLY PREDICTIVE OF DEATH WITHIN 30 DAYS OF PALLIATIVE INTRACRANIAL RADIATION THERAPY COMPLETION FOR METASTATIC DISEASE

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Introduction: For patients with brain metastases, palliative radiation therapy (RT) has long been a standard of care for improving quality of life and optimizing intracranial disease control. The duration of time between completion of palliative RT and patient death has rarely been evaluated.

Methods: A compilation of two prospective institutional databases encompassing April 2015 through December 2018 was used to identify patients who received palliative intracranial radiation therapy. A multivariate logistic regression model characterized patients adjusting for age, sex, admission status (inpatient versus outpatient), Karnofsky Performance Status (KPS), and radiation therapy indication.

Results: 136 consecutive patients received intracranial palliative radiation therapy. Patients with baseline KPS < 70 (OR=2.2; 95%CI= 1.6-3.1; $p<0.0001$) were significantly more likely to die within 30 days of treatment. Intracranial palliative radiation therapy was most commonly delivered to provide local control (66% of patients) or alleviate neurologic symptoms (32% of patients), and was most commonly delivered via whole brain radiation therapy in 10 fractions to 30 Gy (38% of patients). Of the 42 patients who died within 30 days of RT, 31 (74%) received at least 10 fractions.

Conclusions: Our findings indicate that baseline KPS < 70 is independently predictive of death within 30 days of palliative intracranial RT, and that a large majority of patients who died within 30 days received at least 10 fractions. These results indicate that for poor performance status patients requiring palliative intracranial radiation, hypofractionated WBRT courses should be strongly considered.

Translational/Clinical Research ***Resident Physician***

POSTER #125

A NOVEL PROXIMAL CULTURE METHOD FOR STUDYING HETEROTYPIC PARACRINE SIGNALING BETWEEN CANCER CELLS AND THEIR MICROENVIRONMENT

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Paracrine or juxtacrine signaling mediates intracellular interactions that are relevant for many biological processes such as immune responses, angiogenesis, tumor progression, and development. Co-culture and condition medium studies are the most widespread methods to differentiate between these two types of cellular interactions. However, conditioned medium does not recapitulate the effect of localized high concentrations of secreted factors in the microenvironment during paracrine interactions and thus, may lead to inaccurate conclusions. Moreover, it is an acute treatment as compared to the dynamic exchange of factors that occurs during paracrine signaling. To resolve these issues, we have designed a proximal culture method to analyze paracrine signaling. The two cell types are grown on the upper and lower surfaces of a 10 μm-thick polycarbonate membrane with 0.4 μm pores. This allows for cellular communication through the dynamic exchange of secreted factors at localized concentrations while inhibiting juxtacrine signaling. The cells can be collected at the endpoint to determine the effects of paracrine signaling. Since the proximal culture method allows for localized concentration gradients of secreted factors, this method is effective at studying methods that utilize inhibitors or which involve prolonged periods of incubation. This method is primarily used by us to study the cross talk between ovarian cancer cells and mesothelial cells during metastasis; however, it can be extended to any two cell types for the application of paracrine signaling in immunology, tumor microenvironment, and development.

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

POSTER #126

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Basic Science ***Core Facility***

POSTER #127

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POSTER #128

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POSTER #129

IN VIVO THERAPEUTICS CORE

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The In Vivo Therapeutics Core (IVTC) is a state-of-the-art shared resource of the Indiana University Melvin and Bren Simon Cancer Center (IUSCC) that serves as a recognized shared resource of the Indiana University School of Medicine (IUSOM), providing cost-effective and comprehensive services including, but not limited to, on-site breeding facilities as well as a numerous *in vivo* pharmacology models to facilitate the development and testing of novel pharmacological & cellular therapies. It is a certified shared resource of the Indiana Clinical and Translational Sciences Institute (iCTSI). The IVTC maintains multiple mouse breeder colonies on campus.

The Core maintains multiple IACUC-approved protocols dedicated to *in vivo* animal studies. If needed, the IVT Core can work with the Principal Investigator to construct and submit their animal study amendment.

Basic Science ***Core Facility***

POSTER #130

ANGIO BIOCORE

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Basic Science ***Core Facility***

POSTER #131

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POSTER #132

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Basic Science ***Core Facility***

POSTER #133

SUSAN G. KOMEN TISSUE BANK AT IU SIMON CANCER CENTER

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Basic Science ***Core Facility***

POSTER #134

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Basic Science ***Core Facility***

POSTER #135

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Basic Science