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POSTER #1

ENHANCING NAB-PACLITAXEL CHEMOTHERAPY RESPONSE BY MECHANISTICALLY DIVERSE ANTIANGIOGENIC AGENTS IN PRECLINICAL MODELS OF GASTRIC CANCER

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Purpose: Gastric adenocarcinoma (GAC) remains the third most common cause of cancer deaths worldwide. Systemic chemotherapy is commonly recommended as a fundamental treatment for metastatic GAC, however, standard treatment has not been established yet. Angiogenesis plays a crucial role in the progression and metastasis of GAC. We evaluated therapeutic benefits of mechanistically diverse antiangiogenic agents in combination with *nab*-paclitaxel, a next-generation taxane, in preclinical models of GAC.

Experimental Design: Animal survival studies were performed in peritoneal dissemination models in NOD/SCID mice. Tumor growth studies were performed in subcutaneous xenografts in NOD/SCID mice using GAC cell lines or patient-derived xenografts. The mechanistic evaluation involved IHC and Immunoblot analysis in tumor samples.

Results: *Nab*-paclitaxel increased animal survival that was further improved by the addition of antiangiogenic agents ramucirumab (or its murine version DC101), cabozantinib and nintedanib. In a peritoneal dissemination model using MKN-45 GAC cells, median survival in the control group mice (PBS treated) was 21 days after the start of therapy. In comparison with control animals, there was no increase in median survival in the oxaliplatin (21 days), ramucirumab (21 days) or cabozantinib (21 days) therapy groups, but survival was significantly longer in the nintedanib group (26 days, a 24% increase). Animal survival was markedly increased by single-agent *nab*-paclitaxel treatment (40 days, a 90% increase), and was further increased by addition of antiangiogenic agents: NPT+Ram (48 days, a 129% increase), NPT+Cab (69 days, a 229% increase) and NPT+Nin (100 days, a 376% increase). In cell-derived subcutaneous xenografts, *nab*-paclitaxel reduced tumor growth while all three antiangiogenic agents enhanced this effect, with nintedanib demonstrating the greatest inhibition. Furthermore, in GAC patient-derived xenografts (PDX) *nab*-paclitaxel, nintedanib or their combination also delayed tumor growth. The net increase in tumor size was 599 mm^3 in controls, 204 mm^3 in NPT, 211 mm^3 in Nin and -24 mm^3 (tumor regression) in NPT+Nin. Tumor tissue analysis revealed that ramucirumab and cabozantinib only reduced tumor vasculature, while nintedanib in addition significantly reduced tumor cell proliferation and increased apoptosis.

Conclusions: Effects of *nab*-paclitaxel, a promising chemotherapeutic agent for GAC, can be enhanced by new-generation antiangiogenic agents. Among the different agents tested, the *nab*-paclitaxel combination with nintedanib, a new-generation triple angiokinase inhibitor, is most effective in these preclinical gastric cancer models. These results strongly support the therapeutic potential of *nab*-paclitaxel combination with multitarget antiangiogenic agents for improving clinical outcome of gastric cancer patients.

Basic Science ***Faculty***

POSTER #2

PHOSPHATIDYLINOSITOL TRANSFER PROTEINS REGULATE MEGAKARYOCYTE TGF-BETA1 SECRETION AND HEMATOPOIESIS IN MICE

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There are still unknowns regarding homeostatic regulation of hematopoietic stem (HSC) and progenitor (HPC) cells. Deciphering these processes is important for understanding and treating hematopoietic diseases. Phosphatidylinositol is a rare membrane structure lipid that is critical for cellular signaling upon phosphorylation by lipid kinases to generate phosphoinositide. While phosphoinositide pathways contribute to events linked to the cytoskeleton, little is known of these pathways in regulating hematopoiesis. Critical to this pathway are phosphatidylinositol transfer proteins (PITP) that *in vitro* enhance the transfer of aqueous insoluble phosphatidylinositol from one membrane to another. Class I PITP proteins PITPa and PITPb are highly conserved, small, and ubiquitously expressed in mammalian cells. We hypothesized that megakaryocyte (MK) phosphoinositide signaling contributes to HSC and HPC cell regulation. Bone marrow (BM) from conditional knockout mice lacking phosphatidylinositol transfer protein(s) (PITP)a or both PITPa and PITPß specifically in MK and platelets manifested decreased HSC, megakaryocyte-erythrocyte progenitors, cycling HPC (CFU-GM, BFU-E, CFU-GEMM) and significantly reduced engrafting capability as demonstrated through competitive transplantation and limiting dilution analysis. Conditioned media (CM) from thrombopoietin (TPO)-cultured *pitpa*$^{-/-}$and *pitpa*$^{-/-}$/ß$^{-/-}$ BM MK suppressed colony formation of wild type C57Bl/6 BM HPC. The CM from *pitp*$^{-/-}$ BM MK culture contained high levels of transforming growth factor-beta 1 (TGF-ß1) and interleukin (IL)-4, and colony suppression was blocked *in vitro* by neutralizing anti-TGF-ß antibody. Moreover, the BM microenvironment of *pitpa*$^{-/-}$/ß$^{-/-}$ mice had higher concentrations of TGF-b1than their littermate controls upon examination of the BM flush fluid. Treatment of *pitpa*$^{-/-}$/ß$^{-/-}$ mice *in vivo* with anti-TGF-ß antibodies completely restored BM HSC and HPC numbers. *Ex vivo* production of the phosphatidylinositol-derived second messenger inositol trisphosphate (IP_3) was reduced in *pitpa*$^{-/-}$/ß$^{-/-}$ TPO-generated MK. By pharmacologically increasing intracellular calcium in *pitpa*$^{-/-}$/ß$^{-/-}$ MK, TGF-ß over-secretion was reverted. These findings link MK phosphoinositide signaling with homeostatic regulation of hematopoiesis through controlled release of TGF-ß1 and possibly IL-4.

Basic Science ***Faculty***

POSTER #3

ENHANCEMENT OF NAB-PACLITAXEL RESPONSE BY INHIBITION OF INSULIN-LIKE GROWTH FACTOR SIGNALING IN EXPERIMENTAL ESOPHAGEAL ADENOCARCINOMA

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Introduction: Esophageal adenocarcinoma (EAC) is the fastest growing cancer in the western world and the overall 5 year survival rate of EAC is below 20 percent. Most patients with EAC present with locally advanced or widespread metastatic disease, where current treatment is largely ineffective. Therefore, new therapeutic approaches are urgently needed. Epidemiological studies have linked obesity with EAC. Insulin-like growth factor (IGF) signaling is an important mediator in obesity-associated EAC. Paclitaxel (PT) has been used in combination with carboplatin as a standard combination therapy for advanced EAC. PT required emulsification with solvents which has resulted in serious adverse effects in patients. Nanoparticle albumin-bound paclitaxel (nab-paclitaxel) is an albumin-stabilized, cremophor-free and water soluble nanoparticle formulation of PT. Nab-paclitaxel has recently shown greater efficacy over PT in EAC. In this study, we evaluated the improvement in nab-paclitaxel response by addition of BMS-754807, a small molecule inhibitor of IGF-1R/IR signaling, in experimental EAC. **Methods:** We first evaluated the phosphorylation status of IGF-1R/IR protein by western blot in a panel of EAC cell lines. BMS-754807and nab-paclitaxel, alone or in combination were tested for effects on cell growth detected by WST-1 assay and on cell apoptosis detected by western blot of cleavage of caspase 3 and PARP. We then explored the antitumor efficacy with survival advantage following BMS-754807and nab-paclitaxel monotherapy and in combination in murine subcutaneous xenograft and peritoneal metastatic survival models of human EAC. **Results:** BMS-754807 dose dependently inhibited in-vitro cell proliferation of EAC cell lines having phosphorylation of IGF-1R/IR protein and interestingly the addition of IC25 dose of BMS-754807 significantly decreased the nab-paclitaxel IC50. The co-administration of BMS-754807 and nab-paclitaxel effectively enhanced cleavage of caspase-3 and PARP. BMS-754807 in combination with nab-paclitaxel treatment resulted in significantly higher antitumor efficacy and survival benefit compared with BMS-754807 or nab-paclitaxel treatment alone.In subcutaneous xenografts using OE19 cells, average net tumor growth after two weeks in different therapy groups was 558.67 mm^3in control, 208.47 mm^3after BMS-754807 ($p=0.043$), 104.60 mm^3after nab-paclitaxel ($p=0.013$), and 14.30 mm^3after BMS-754807 plus nab-paclitaxel ($p=0.0005$). There was a significant increase in median animal survival after BMS-754807 plus nab-paclitaxel treatment (85 days) compared to control (47 days, $p=0.0034$), to BMS-754807 therapy (57 days, $p=0.0021$) or to nab-paclitaxel (68 days, $p=0.0339$) therapy. **Conclusion:** These results support the potential of BMS-754807 in combination with nab-paclitaxel as an effective option for EAC therapy.

Basic Science ***Faculty***

POSTER #4

SYNERGISTIC EFFECTS OF FORETINIB WITH LAPATINIB IN MET AND HER2 CO-ACTIVATED EXPERIMENTAL ESOPHAGEAL ADENOCARCINOMA

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Introduction: Recent studies have demonstrated that HER2 and MET receptor tyrosine kinases are co-overexpressed in a subset esophageal adenocarcinoma (EAC). We therefore studied the usefulness of combining HER2 and MET targeting by small-molecule inhibitors foretinib and lapatinib both in-vitro and in-vivo models of experimental EAC. **Methods**: In this study we first characterized MET and HER2 activation in a panel of human EAC cell lines and the differential susceptibility of these EAC cell lines to single agents or combinations of foretinib, a multi-kinase MET inhibitor, with HER2 targeted agent lapatinib. We evaluated the levels of phosphorylation status of MET and HER2 proteins using western blot analysis in EAC cell lines. Foretinib and lapatinib, as single agent or in combination were tested for effect on cell growth as detected by WST-1 assay and on cell apoptosis as detected by western blot analysis of cleavage of caspase 3 and poly ADP ribose polymerase (PARP). In addition, we explored the antitumor efficacy with survival advantage following foretinib and lapatinib monotherapy and in combination in murine subcutaneous xenograft and peritoneal metastatic survival models of human EAC. **Results:** The OE33 EAC cell line with phosphorylation of both MET and HER2, demonstrated reduced sensitivity to foretinib and lapatinib when used as a single agent. The co-administration of foretinib and lapatinib effectively inhibited both MET and HER2 phosphorylation, synergistically inhibited cell growth and induced apoptosis, overcoming single agent resistance. In the OE19 EAC cell line with only HER2 phosphorylation and the ESO51 EAC cell line with only MET phosphorylation, profound cell growth inhibition with induction of apoptosis was observed in response to single agent foretinib and lapatinib, respectively, with lack of enhanced growth inhibition when the two drugs were combined. Foretinib in combination with lapatinib treatment resulted in significantly higher antitumor efficacy and survival benefit compared with foretinib or lapatinib treatment alone.In subcutaneous xenografts using OE33 cells, average net tumor growth after two weeks in different therapy groups was 247.83 mm^3in control, 216.71 mm^3after foretinib (p=0.49), 239.68 mm^3after lapatinib (p=0.74), and 108.06 mm^3after foretinib plus lapatinib (p=0.0011). There was a significant increase in median animal survival after foretinibplus lapatinib treatment (71 days) compared to control (60 days, p=0.0021), to foretinib therapy (63 days, p=0.0019) or to lapatinib (61 days, p=0.0019) therapy. **Conclusion:** These data suggest that combination therapy with foretinib and lapatinib should be tested as a treatment option for HER2 positive patients with MET-overexpressing EAC. Therefore, this combination therapy could be a novel treatment strategy for EAC with MET and HER co-activation.

Basic Science ***Faculty***

POSTER #5

GENOME-WIDE DNA LESION ANALYSIS

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Genomic DNA is under constant environmental abuse, generating various lesions. These lesions can change the nucleobase complementarity resulting in mutations after replication. The accumulation of mutations in a cell may eventually alter the cellular behavior, leading to uncontrolled cell growth and division, a hallmark of cancer.

Solar irradiation and oxidative stress are two most common types of environmental stresses cells encounter. Oxidative stress is also a common side effect in chemotherapy. Under oxidative conditions, guanine residues in DNA can be readily modified to 8-oxoguanine (8-oxo-G), which is widely regarded as a biomarker of oxidative stress. Under solar irradiation, the thymine residue is the most reactive nucleobase. Our data indicate that 5-thyminyl-5,6-dihydrothymine, i.e. the spore photoproduct (SP), can be formed in humans in a relatively high yield. The presence of SP thus may play an important role in the occurrence of skin cancer. It is therefore of paramount importance to develop assays for reliable analysis of these two DNA lesions.

For the 8-oxo-G analysis, our group has chemically synthesized the unlabeled 8-oxo-G and prepared 8-oxo-G containing oligonucleotides. We will also synthesize ^{18}O labeled 8-oxo-G as an internal standard for 8-oxo-G quantification via LC/MS assays. Moreover, Burrows et al. have used $IrBr_6^{2-}$ to achieve the selective oxidation of 8-oxo-G, which subsequently allows labeling and enrichment of 8-oxo-G containing genome fragments for high throughput sequencing analysis. We are currently working to repeat this assay for 8-oxo-G mapping in the genome.

For the SP analysis, we have conducted a 14-step synthesis to prepare the dinucleotide SP. Moreover, we have prepared tri-deuterium labeled d_3-SP via a photochemical reaction that serves as the internal standard for SP quantification via LC/MS assays. To enable SP genomic mapping studies, we have chemically conjugated an SP-containing oligonucleotide to a carrier protein and then used the conjugate as the antigen for mouse immunization to produce a monoclonal SP antibody. This antibody allows the SP enrichment via immunoprecipitation and the mapping of SP distribution in the genome via high throughput sequencing.

These studies, when coupled with bioinformatics analysis of the mutational data obtained from cancer patients, may allow us to reveal the roles of DNA lesions in cancer induction as well as cancer relapse. Moreover, our research described here may provide a general approach to the field for the investigation of other DNA lesions.

Basic Science ***Faculty***

POSTER #6

COMPREHENSIVE CHARACTERIZATION OF CACHEXIA IN THE KPC GENETICALLY ENGINEERED MOUSE MODEL OF PANCREATIC DUCTAL ADENOCARCINOMA

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Pancreatic ductal adenocarcinoma (PDAC) is among the most lethal of malignancies. Patients with PDAC have the highest risk of developing cachexia, or loss of fat and muscle, with associated fatigue and dysmobility. Therapeutics designed to prevent or slow cachexia have the potential to improve quality of life and survival for this devastating disease. Activin is elevated in the circulation of patients with PDAC cachexia. Inhibition of Activin receptor signaling has been trialed for cachexia in patients with pancreatic cancer, with unclear benefit. Herein, we examined a related strategy using ACVR2B/Fc, a soluble receptor-chimera trap for Activins and related proteins, to prevent loss of skeletal muscle mass in the genetically engineered mouse model of PDAC, KPC (LSL-KrasG12D;LSL-Trp53R172H;Pdx1-Cre). KPC mice have been widely used to examine molecular pathways and test cancer therapies but as yet has not been comprehensively characterized for cachexia over the course of spontaneous tumor initiation and growth. Here we generated KPC mice versus genotype controls and monitored tumor formation and changes in body weight (BW) and EchoMRI-assessed body composition up to 30 weeks of age. PDAC tumor development in KPC mice had a median latency of 18 weeks of age. We observed that KPC mice ceased BW gain at approximately 17 weeks of age followed by a progressive BW decline, while the controls steadily gained BW, leading overall to a maximum BW loss of 44% in KPC by 30 weeks. KPC mice also demonstrated reduced fat and carcass mass. In contrast to other organs, spleen weight increased in KPC mice. Although patients with PDAC exhibit diabetes, random blood glucose over a period of 22 weeks was not increased in KPC mice, and, in fact, tended to be lower than in controls. This suggests that PDAC may not induce wasting through diabetic mechanisms or that glucose metabolism might be intact in PDAC cachexia. ACVR2B/Fc treatment prevented weight loss, muscle loss and reduced fat wasting, but also further enlarged the spleen, although tumor growth was not significantly inhibited by ACVR2B/Fc. Activation of known catabolic signals including FOXO1, SMAD3, STAT3 were observed in KPC muscle as well as their downstream markers of protein catabolism, including activation of the ubiquitin-proteosome pathway, inhibition of translation, and activation of autophagy. Most of these were reduced by ACVR2B/Fc. Finally, RNA-sequencing of the skeletal muscle from KPC mice euthanized at early or late cachexia, with and without ACVR1B/Fc-treatment, revealed many differentially expressed genes in KPC vs matched controls. Further analysis is underway. In summary, the KPC model recapitulates many of the phenotypic and genotypic features of the human cancer cachexia and could be used to identify cancer cachexia-targeting therapies. The model could be particularly useful for experiments intended to prevent early cancer cachexia.

Basic Science ***Faculty***

POSTER #7

PHARMACOLOGICAL INHIBITION OF FGFR SIGNALING MODULATES PULMONARY IMMUNE MICROENVIRONMENT

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Cancer metastasis is the main cause of deaths in breast cancer (BC) patients. As such development of novel cancer therapeutics to treat metastatic lesions is of great importance to reduce patients' suffering. During cancer progression, primary tumor cells hijack a normal cellular process termed epithelial to mesenchymal transition (EMT) to metastasize to distant sites. Although the contributions of EMT to metastatic progression are well understood, the goal of developing durable treatments targeting EMT driven cancer is yet to be met. Previously, we have established that over-activation of fibroblast growth factor receptor 1, FGFR1, is a stable marker of EMT in metastatic settings and inhibition of FGFR signaling via novel covalent kinase inhibitor (FIIN4) can prevent pulmonary metastasis in highly aggressive metastatic mouse models. Though such treatment with FIIN4 significantly increases the overall survival of the mouse; eventually, they succumb to the disease. The goal of the current project is to increase the efficacy of FIIN4 to treat metastatic cancer. Interestingly, we observed that FIIN4 treatment leads to significant increase in survival of metastasis bearing immune competent mice (BALB/c) in compared to immune deficient ones (NRGs). Next, flow cytometry and immunohistochemistry analyses have revealed that FIIN4 treatment modulates the pulmonary immune microenvironment by increasing the presence of CD8+ T cells, reducing the number of CD4+ T cells and immune suppressive myeloid derived suppressor cells (MDSCs). Importantly, FIIN4 treatment did not also the number of macrophages. Interestingly, remaining MDSCs post FIIN4 treatment in the pulmonary tissues were highly immune suppressive as evident by very high-level expression of PDL1 ligand. Subsequently, we pursued the combination therapy using FIIN4 and immune check point therapies (anti-PDL1) to treat cancer metastasis. Currently, we are investigating the mechanism of differential CD8+ T cell recruitments in the lung following FIIN4 treatments. In addition, we are validating the efficacy of this combination therapy in other aggressive breast cancer models.

Basic Science ***Graduate Student***

POSTER #8

BIASED SIGNALING DOWNSTREAM OF EPIDERMAL GROWTH FACTOR RECEPTOR REGULATES PROLIFERATIVE VERSUS APOPTOTIC RESPONSE TO LIGAND

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Inhibition of EGFR signaling by kinase inhibitors and monocloncal antibodies has proven effective in the treatment of multiple cancers. In contrast, metastatic breast cancers (BC) derived from EGFR-expressing mammary tumors are inherently resistant to EGFR-targeted therapies. Mechanisms that contribute to this inherent resistance remain poorly defined. Here we show that in contrast to primary tumors, ligand-mediated activation of EGFR in metastatic BC is dominated by STAT1 signaling. This change in downstream signaling leads to apoptosis and growth inhibition in response to EGF in metastatic BC cells. Mechanistically, these changes in downstream signaling result from an increase in the internalized pool of EGFR in metastatic cells, increasing physical access to the nuclear pool of STAT1. Inhibition of endosomal signaling using an EGFR inhibitor linked to a nuclear localization signal specifically prevents EGF-induced STAT1 phosphorylation and cell death, without affecting EGFR:ERK1/2 signaling. Pharmacologic blockade of ERK1/2 signaling through the use of the MEK1/2 inhibitor, trametinib, dramatically biases downstream EGFR signaling toward a STAT1 dominated event, resulting in enhanced EGF-induced apoptosis in metastatic BC cells. Importantly, combined administration of trametinib and EGF also facilitated an apoptotic switch in EGFR-transformed primary tumor cells, but not normal mammary epithelial cells. These studies reveal a fundamental distinction for EGFR function in metastatic BC and demonstrate that pharmacological biasing of EGFR signaling toward STAT1 activation is capable of revealing the apoptotic function of this critical pathway.

Basic Science ***Graduate Student***

POSTER #9

ABCG2 EXPRESSION CONTRIBUTES TO RESISTANCE OF CC-115, A DUAL MTOR/DNA-PK INHIBITOR

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Introduction: A major obstacle in the treatment of cancers is innate or acquired resistance. Multi-drug resistance proteins such as MDR1, ABCG2, MRP1, and LRP have been shown to be involved in resistance and are predictors of clinical outcomes. Expression of ABCG2, an ATP-binding cassette efflux transporter, may be responsible for observed variability of the efficacy of CC-115, a dual mTOR/DNA-PK inhibitor, currently in clinical trials. Here we evaluate the role of ABCG2 in CC-115 resistance.

Methods: Western blots, methylene blue and MTT assays, and FACS were used to determine ABCG2 expression, cell survival/viability with and without inhibitors, and accumulation of CC-115 respectively.

Results:MCF7/AdVp3000 (M3K) cells have been shown to have a significant increase in ABCG2 as compared to parental MCF7 cells. M3K cells had a 50-fold increase in IC50 to CC-115 compared to MCF7. Use of ABCG2 inhibitors FTC and C8 (1uM), decreased the IC50 of CC-115 in M3K cells by 10-fold. Accumulation studies using FACS showed that M3K cells had significantly lower accumulation of CC-115 as compared to MCF7 and addition of FTC and C8 (1uM) significantly enhanced accumulation of CC-115 to the same extent as seen in MCF7 cells. Next, HEK293/ABCG2 stable clone with overexpression of ectopic ABCG2 and its control HEK293/vec cells were used to determine ABCG2's direct impact on CC-115 resistance. HEK293/ABCG2 cells had a 10-fold increase in resistance to CC-115 with an IC50 of 2.05 uM as compared to 0.23 uM in HEK293/vec cells. Addition of FTC and C8 (1uM) decreased the IC50 by 10-fold in HEK293/ABCG2 cells. Accumulation of CC-115 in HEK293/ABCG2 cells was also significantly lower than that in HEK293/vec cells and addition of FTC and C8 (1uM) significantly increased its accumulation only in HEK293/ABCG2 cells.

Discussion: The results from this study suggest that ABCG2 contributes to and possibly is responsible for CC-115 resistance and that inhibiting ABCG2 could lead to reversal of CC-115 resistance, increased response rate, highlights the importance of personalized therapies, and has the potential to increase patient survival.

Basic Science ***Graduate Student***

POSTER #10

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 relapse, 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. In particular, members of the ALDH1A subfamily oxidize retinal to retinoic acid as part of the retinoic acid signaling pathway. More specifically, ALDH1A1 is often thought to be the dominant ALDH1A 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 analogues. An X-ray crystallography structure was determined for each scaffold bound to ALDH1A1 in order to identify the mechanism of binding and inform the design of future compounds. Inhibitors from both series were shown to inhibit proliferation of multiple 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, 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. The anti-proliferative effect of the compounds could be partially rescued by two downstream effectors of the retinoic acid pathway: All-trans retinoic acid, a Retinoic Acid Receptor (RAR) ligand, and Rosiglitazone, a Peroxisome Proliferator-Activated Receptor ¿ (PPAR¿) activator. The ability to rescue the effect of ALDH1A1 inhibitors suggests that they engage the intended target and the inhibit the retinoic acid pathway 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 #11

NOVEL CORRELATION-BASED NETWORK ANALYSIS OF BREAST TUMOR METABOLISM IDENTIFIES THE GLYCEROL CHANNEL PROTEIN AQUAPORIN-7 AS A REGULATOR OF BREAST CANCER PROGRESSION

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The complex yet interrelated connections between cancer metabolism, gene expression, and oncogenic driver genes have the potential to identify novel biomarkers and drug targets with prognostic and therapeutic value. Using GC-MS, LC-MS/MS, and capillary zone electrophoresis (CZE)-MS platforms, we quantified and compared the levels of 374 metabolites in breast tumor tissue from normal tissue and transgenic mouse breast cancer models overexpressing a panel of oncogenes (PyMT, PyMT-DB, Wnt1, Neu, and C3-TAg). Comparison of the metabolite profiles identified oncogene-induced metabolic reprogramming of the tumor tissues.

To develop a higher order understanding of the driver genes and metabolites in breast cancer, we next developed a correlation-based network analysis that captured interactions between both metabolite and gene expression data. Through this analysis, we uniquely identified a metabolic network of metabolites and genes that have prognostic value in breast cancer patients. Our network analysis identified 35 metabolite and 33 gene hubs that had the most network correlations. The gene and metabolite hubs identified by correlated network analysis are likely integral to breast tumor metabolism.

We began investigating the role of the hubs during breast cancer and initially focused on the gene hub aquaporin-7 (Aqp7), a water and glycerol channel protein as a novel regulator of breast cancer. We discovered that AQP7 is a prognostic marker of overall survival and metastasis in breast cancer patients. By cell-based assays, we discovered that in normal mouse mammary epithelial cells, reduced expression of AQP7 inhibits contact inhibition, increases migration, and promotes increased branching in 3-D collagen culture models of mammary branching. In metastatic mouse breast cancer cell lines, reduced expression of AQP7 decreases proliferation, and significantly decreases primary tumor burden as well as the number of metastatic tumors in the lung *in vivo*. Furthermore, metabolomics on AQP7 knockdown cells and tumors reveal significantly altered lipid levels and redox pathways.

Using an unbiased, discovery-based approach, this study shed light on important players in breast cancer metabolism from a new perspective that complements current guided network analyses. This study further identifies AQP7 as a novel regulator of breast cancer progression, likely through altering cellular redox metabolism and lipid metabolism.

Basic Science ***Graduate Student***

POSTER #12

REGULATION OF THE ONCOGENE ZNF217 PROMOTES BREAST CANCER METASTASIS TO LUNG

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The oncogene and transcription factor ZNF217 is overexpressed in 20-30% of breast cancers. Its overexpression correlates strongly with poor prognosis in patients and causes accelerated tumor progression, metastasis, and chemoresistance *in vivo*. While several studies have examined the regulation of ZNF217 at the gene and mRNA levels, little is known about how ZNF217 protein is regulated. In this study we identify the regions of ZNF217 that are required for protein turnover and breast cancer, including increased invasion, tumor burden, and metastasis. Due to the high expression and aberrant localization of ZNF217 in some human breast tumors, ZNF217 protein expression levels and localization may be critical determinants of ZNF217's function during breast cancer *in vivo*.

To investigate the role of ZNF217 protein on cancer progression, we first examined ZNF217 protein expression in human breast tumors by western analysis. Besides full length ZNF217 protein, smaller ZNF217 proteins were also detected and were even more prominent than full length ZNF217 in both human breast tumors and cell lines, but the importance of these smaller isoforms remains unknown. To investigate the function of these smaller ZNF217 proteins and to determine the regions of the ZNF217 protein that are required to promote breast cancer, we examined the consequences of removing regions of ZNF217 by expression of truncation mutants or full length ZNF217 in human breast cancer cells. We then tested these mutants for their oncogenic potential by both cell culture and *in vivo* experiments. We tested the mutants for their ability to promote phenotypes usually associated with ZNF217 overexpression, including increased branching of organoids, protein turnover, tumor burden, and metastasis. This approach identified the regions of ZNF217 required for oncogenic potential. Both the N-terminus and C-terminus of ZNF217 were required for organoid branching. In addition, expression of truncated ZNF217 in breast cancer cells significantly increased tumor burden in orthotopic mammary gland transplantation experiments and increased metastatic tumor burden *in vivo* by tail vein injection. Together, these results suggest that the post-translational regulation of ZNF217 protein may be clinically valuable as a potential prognostic marker of advanced metastatic breast cancer.

Basic Science ***Graduate Student***

POSTER #13

THE ROLE OF THE INNATE IMMUNE RESPONSE IN ERG-POSITIVE 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, controlling 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.

We also began investigating the mechanism by which ERG's protein stability is regulated. At high concentrations, we noticed that the inhibitor causes the loss of ERG protein. While the literature reports ERG protein stability is regulated through an N-terminal degron, we have observed a loss of an ERG truncation mutant that lacks this degron. By utilizing the translation inhibitor, cycloheximide, we have shown this loss of ERG protein is due to diminished protein stability. We then showed ERG is degraded through the proteasome by rescuing protein expression via proteasomal inhibition. We are currently cloning various deletions within ERG to pinpoint the region regulating this protein degradation.

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. A treatment that targets ERG could limit the metastatic potential of early stage cancer and reduce the aggressiveness of late stage cancers.

Basic Science ***Graduate Student***

POSTER #14

PRMT5-MEDIATED METHYLATION OF YBX1 REGULATES NF-¿B ACTIVITY IN COLORECTAL CANCER

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Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the US with a staggeringly less than 14% survival rate for patients with metastatic disease. This poor clinical outcome for CRC patients is largely attributed to the lack of understanding of the factors that drive CRC progression, leading to a dearth of available treatment strategies for this disease. Today, treatment modalities and clinical management of colorectal cancer (CRC) are fundamentally based on the success of controlling specific tumorigenic pathways. Interestingly, aberrant activation of the transcription factor nuclear factor ¿B (NF-¿B) is increasingly recognized as a crucial player in CRC progression. Therefore, understanding the mechanisms underlying regulation of NF-¿B holds great promise for devising new therapeutic strategies for CRC. Recently, we identified the oncogenic protein, Y-box binding protein 1 (YBX1), as a novel activator of NF-¿B. Since YBX1 overexpression has also been linked to poor patient outcome in CRC, we sought to further understand whether this YBX1/NF-¿B axis could be a potential target for CRC treatment. In this study, we discovered that YBX1 is methylated on arginine 205 (YBX1-R205me2), an event that is critical for YBX1-mediated NF-¿B activation and target gene expression. Additionally, co-immunoprecipitation studies revealed that the R205 to alanine (A) mutant (YBX1-R205A) significantly diminished the interaction between YBX1 and the p65 subunit of the transcriptionally active NF-¿B, demonstrating a novel mechanism by which methylation of YBX1 mediates protein-protein interactions. Overexpression of YBX1-R205A significantly attenuated the migration, proliferation, and anchorage-independent growth of a panel of CRC cells, suggesting that YBX1-R205me2 is essential to the oncogenic functions exerted by the YBX1/NF-¿B axis in CRC. Furthermore, we revealed that protein arginine methyltransferase 5 (PRMT5) is responsible for the methylation of YBX1-R205. Collectively, our novel findings present a complex picture of the sophisticated regulation of NF-¿B through PRMT5-mediated YBX1-R205 methylation and suggest that pharmacological disruption of the YBX1/NF-¿B axis using PRMT5 inhibitors could serve as the basis for new therapeutics that impede YBX1/NF-¿B-driven CRC progression.

Basic Science ***Graduate Student***

POSTER #15

PAK1 INHIBITION REDUCES TUMOR SIZE AND EXTENDS THE LIFESPAN OF ANIMALS IN A GENETICALLY ENGINEERED MOUSE MODEL OF NEUROFIBROMATOSIS TYPE 2 (NF2)

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Neurofibromatosis Type 2 (NF2) is an autosomal dominant cancer predisposition syndrome in which patients develop multiple tumors of neural crest derived origin, most commonly bilateral vestibular schwannomas. Oncogenic transformation in this disease appears to be driven by the loss of heterozygosity at the *NF2* locus wherein patients are born with a single functional copy of *NF2* which is then subsequently sporadically lost leading to complete loss of the protein in which it encodes, Moesin-Ezrin-Radaxin like protein (Merlin). Vestibular schwannomas also can develop spontaneously in patients without germline mutations in *NF2*, and greater than 90% of these sporadic tumors harbor biallelic disruption of *NF2*, suggesting that Merlin plays a critical function as a tumor suppressor in Schwann cells. Although relatively little is known about how Merlin functions as a tumor suppressor, *in vitro* studies have shown that Merlin can act as a negative regulator of the group A serine/threonine p21 activated kinase, PAK1. *PAK1* is a known oncogene which serves as a critical signaling node regulating cell proliferation, evasion of apoptosis, and DNA damage repair and is commonly amplified in a variety of human malignancies including up to a third of breast cancers. PAK1 has significantly increased basal activity in Merlin deficient Schwann cells and we therefore hypothesized that Merlin is a critical negative regulator of PAK1 in Schwann cells and that loss of Merlin leads constitutive activation of PAK1, which in turn drives oncogenic transformation and tumor growth.

To test our hypothesis, we globally deleted *Pak1* in our previously published *Nf2*-cKO model of NF2. Deletion of *Pak1* significantly reduced the sensorineural hearing loss and average tumor size and significantly increased the 15 month survival of our *Nf2*-cKO animals. In an attempt to move this finding translationally we worked with CPAC to optimize a previously shelved, potent and specific PAK1 small molecule inhibitor for a therapeutic trial in our mice. In a six week dose escalation, the compound was tolerated at doses sufficient to significantly reduce PAK1 activation in tumor bearing tissues in the mice. We have proceeded to an efficacy trial in the mice. There are currently no approved chemotherapeutics for the treatment of NF2, and inhibition of PAK1 is of therapeutic interest in other malignancies. Therefore, these data showing small molecule inhibition of PAK1 *in vivo* in endogenous tumor tissue could be a significant step forward in the treatment of an orphan disease as well as a variety of common solid tumors.

Basic Science ***Graduate Student***

POSTER #16

POTENTIAL ROLES FOR SPT4 IN RNA POLYMERASE II PROCESSIVITY AND NEURODEGENERATIVE DISORDERS

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Several neurodegenerative disorders including Huntington disease (HD), spinocerebellar ataxia, amyotrophic lateral sclerosis (ALS), and frontotemporal dementia (FTD) are characterized by expansions of nucleotide repeats. When these genomic repeat expansions are transcribed and/or translated, they can lead to cellular toxicity through a variety of possible mechanisms such as, RNA foci formation (3-5), protein aggregation, and loss of normal protein function.ALS and FTD are both commonly caused by a hexanucleotide repeat (GGGGCC) expansion in the *C9orf72* gene. Previous therapeutic approaches have attempted to use antisense oligonucleotides (ASOs) to target expanded GGGGCC repeat mRNA, but the repeat is also transcribed in the antisense direction, and the antisense repeat mRNA is also known to accumulate and form foci in ALS and FTD. Recent work has found that the transcription factor Spt4 plays a role in the transcription of these genomic repeat expansions, both sense and antisense, and that its knockout or knockdown mitigates the phenotypic effects of the expansion repeats.

Spt4 is part of the DSIF transcription elongation complex along with Spt5, thought to have roles in RNA Polymerase II (RNAPII) processivity, heterochromatin dynamics during transcription, and co-transcriptional mRNA processing. Not much is known about the roles Spt4 specifically plays in regulating transcription elongation, limiting its potential as a novel drug target. My current research goal is to investigate how Spt4 mechanistically regulates transcription elongation, and how the RNAPII interactome and global transcription dynamics alter in the absence of Spt4. The RNAPII complex (with a FLAG-tagged subunit) and any interacting proteins in wildtype or *spt4 knockout* yeast can be isolated and analyzed via an affinity purification mass spectrometry workflow. My preliminary results suggest that in an *spt4 knockout* strain, RNAPII has increased interactions with an early transcription termination complex, and proteins involved in degradation by the proteasome. These results suggest that Spt4 plays a distinct role in RNAPII processivity, and that in its absence, RNAPII is more likely to arrest, and needs to be degraded to be removed from the DNA. Further investigation of the role of Spt4 in transcription elongation will shine light on if Spt4 truly has potential as a therapeutic target for neurodegenerative disorders, and if so, what are the best strategies in which to target it.

Basic Science ***Graduate Student***

POSTER #17

IL-6 FAMILY CYTOKINES PLAY A PROTECTIVE ROLE IN THE ESTABLISHMENT OF STROMAL CELLS IN PANCREATIC DUCTAL ADENOCARCINOMA

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Pancreatic Ductal Adenocarcinoma (PDAC) is the most common form of pancreatic cancer amounting to roughly 80% of cancer cases. PDAC arises in the pancreas with infiltration into surrounding tissues resulting in metastasis. It is characterized by the presence of a dense stroma of fibroblasts and connective tissue and likely involves pancreatic stellate cell recruitment in response to pancreatic injury. Pancreatic cancer is the fourth leading cause of cancer related deaths and the five year survival rate is found to be 5%-7% for cancer patients. Due to tumor heterogeneity and potentially due to the dense, reactive stroma, PDAC is often found to be chemoresistant. Our lab focuses on the interactions of the IL-6 family of cytokines and the progression of pancreatic cancer. Pro-inflammatory cytokines are found in the microenvironment of pancreatic cancer, both in the tumor cells and in the stromal cell compartments. Interleukin-6 (IL-6) is known to promote PDAC development and progression as well as cachexia; however, less is known about Leukemia Inhibitory Factor (LIF). Previous findings in other systems demonstrated that overexpression of LIF has an effect on the expression of IL-6. We sought to determine the roles of IL-6 and LIF in PDAC using CRISPR-mediated deletion in a PDAC cell line derived from mice genetically engineered for pancreatic cancer. Clonal cell lines were validated and injected in the pancreas of C57BL/6 mice. KPC cells deleted for IL-6 (KPC-IL6ko) produced less cachexia and longer survival than wild-type KPC cells (KPC-WT) in orthotopic implantation, and trended towards smaller tumor size. In contrast, KPC cells deleted for LIF (KPC-LIFko) showed enhanced tumor growth and more severe cachexia phenotypes after orthotopic implantation. We hypothesized that tumor-derived IL-6 and LIF might affect establishment of the stromal cell compartment in PDAC. To begin to quantify the stromal cell compartment, tumor sections were stained with Picro Sirius Red to detect collagen I and III fibers, counterstaining with Hematoxylin to detect nuclei. ImageJ was used to quantify staining of 25 random fields from each of the 5 tumors. Our results indicate that KPC-IL6ko tumors showed significantly elevated collagen staining compared to KPC-WT. Our results indicate that IL-6 expression from tumor cells modulates the stromal cell compartment. Deletion of IL-6 from the tumor promoted stromal cell reactivity and reduced disease severity, despite intact IL-6 expression in stromal cells. Thus inhibition of IL-6 in the PDAC micro-environment might be a protective role. Results from KPC-LIFko studies are still pending. Taken together, our data suggest the IL-6 family of cytokines plays an important role in the establishment of stromal cells in PDAC and affects pancreatic cancer homeostasis.

Basic Science ***Graduate Student***

POSTER #18

ENZYME-RESPONSIVE DYNAMIC HYDROGELS FOR STUDYING CELL-MATRIX INTERACTIONS IN PANCREATIC DUCTAL ADENOCARCINOMA

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Statement of Purpose: The tumor microenvironment (TME) governs all aspects of cancer progression and in vitro 3D culture platforms are increasingly developed to emulate the interactions between stromal tissues and cancer cells. To mimic the complex compositions and dynamic changes in gel mechanics of TME, we developed a dynamic gelatin/hyaluronic acid (Gel/HA) hybrid gel system. Gelatin was dually modified with norbornene and 4-hydroxyphenylacetic acid (4-HPA). The former renders this protein photo-crosslinkable and the later affords responsiveness to tyrosinase (TYR)-triggered stiffening. In addition to the modified gelatin that provides basic cell adhesive motifs and protease cleavable sequences, HA, an essential tumor matrix, was covalently incorporated into gels. We characterized macromer modification, gel crosslinking, as well as enzyme-triggered stiffening and degradation. We also evaluated influence of matrix compositions and dynamic stiffening on pancreatic ductal adenocarcinoma (PDAC) cells. **Methods:** Gelatin-norbornene (Gel_{NB}) was modified with 4-HPA via carbodiimide chemistry to yield Gel_{NBHPA}. Gels were prepared by reacting NB moieties of functionalized gelatin (Gel_{NB} or Gel_{NBHPA}) with thiol motifs on PEG4SH or THA through thiol-norbornene photopolymerization. Next, gels were swollen in PBS at 37°C for a day, followed by incubating in TYR solution (1 kU/mL) for 6 hours to achieve in situ stiffening. Gel elastic moduli (G' & G") were measured with oscillatory rheometry in strain-sweep mode. To study the influences of matrix compositions on PDAC cells, COLO-357 cells were encapsulated in modularly crosslinked gels: (1) Gel_{NB} with PEG4SH (Gel/PEG), (2) Gel_{NB} with THA (Gel/HA), (3) Gel_{NBHPA} with PEG4SH (Gel_{NBHPA}/PEG), and (4) Gel_{NBHPA} with THA (Gel_{NBHPA}/HA) (Fig. 1B). TYR-triggered stiffening was performed one day post-encapsulation. Live/dead staining and confocal imaging were used to evaluate cell viability and morphology. At day 14, total RNA was extracted from cell-laden gels for mRNA expression analyses using Taqman® array – PDAC (Housekeeping gene: GAPDH). **Results:** Results of the *in vitro* study showed cell clusters were visibly larger in soft and HA-free gels. However, cluster sizes were significantly smaller when the hydrogels were soft and contained HA or stiffened but contained no HA. Interestingly, the morphology of the clusters in Gel_{NBHPA}/HA gels became highly irregular, suggesting increased cell motility. We evaluated PDAC-related gene expression by TaqMan® Array. We identify genes that were upregulated unique to the presence of HA, to a stiffened microenvironment, or to the combination of both. We also characterized mRNA expression from cells cultured in Gel_{NBHPA}/HA gels. Results showed that these cells underwent epithelial-mesenchymal transition, as evidenced by down regulation of epithelial cell markers, E-cadherin (CDH1) and upregulation of mesenchymal markers, N-cadherin (CDH2), SNAIL-1 and vimentin (VIM).

Basic Science ***Graduate Student***

POSTER #19

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 #20

LINKING LSD1 TO INFLAMMATION-INDUCED EMT

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Aberrant activities of chromatin modifying enzymes have been implicated in the pathogenesis of cancer. Lysine-specific demethylase 1 (LSD1) is a lysine demethylating enzyme that is overexpressed in up to 80% of patients with colorectal-cancer (CRC), and its overexpression promotes enhanced proliferative and migratory phenotypes in CRC cell culture models. We have implicated LSD1 as a potent positive regulator of Akt kinase activation. Activation of Akt enhances proliferative and migratory phenotypes, potentially explaining phenotypes associated with overexpression of LSD1. Interestingly, activation of Akt and Epithelial-mesenchymal transition (EMT), a pathological mechanism underlying metastasis, are both induced by inflammation; inflammation is highly linked to CRC progression. Surprisingly, we have found that inflammation may activate enzymatic activity of LSD1, enabling it to regulate EMT through an Akt-independent mechanism. Studying this process will allow us to identify therapeutic strategies that target oncogenic LSD1 activity both basally and during inflammation.

Basic Science ***Graduate Student***

POSTER #21

OSTEOMACS INTERACT WITH MEGAKARYOCYTES AND OSTEOBLASTS TO REGULATE MURINE HEMATOPOIETIC STEM CELL FUNCTION

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Networking between hematopoietic stem cells (HSC) and cells of the hematopoietic niche is critical for stem cell function and maintenance of the stem cell pool. We characterized calvariae-resident osteomacs (OM) and their interaction with megakaryocytes to sustain HSC function and identified distinguishing properties between OM and marrow-derived macrophages. OM, identified as CD45+F4/80+ cells were easily detectable (3-5%) in neonatal calvarial cells. Co-culture of neonatal calvarial cells with megakaryocytes for 7d increased OM 3-6 fold demonstrating that megakaryocytes regulate OM proliferation. OM were required for the hematopoiesis enhancing activity of osteoblasts and this activity was augmented by megakaryocytes. Serial transplantation demonstrated that the repopulating potential of HSC was best maintained by in vitro cultures containing osteoblasts, OM, and megakaryocytes demonstrating that hematopoiesis is best maintained through collaborative interactions between all three cell types. With or without megakaryocytes, marrow-derived macrophages were unable to functionally substitute for neonatal calvarial cell-associated OM. Similar to marrow-derived macrophages, OM differentiated into multinucleated, TRAP+ osteoclasts capable of bone resorption. Nine-color flow cytometric analysis revealed that although marrow-derived macrophages and OM share many cell surface phenotypic similarities (CD45, F4/80, CD68, CD11b, Mac2, and GR-1), only a subgroup of OM co-expressed MCSF-R1 and CD166, thus providing a unique profile for OM. CD169 was expressed by both OM and marrow-derived macrophages and therefore was not a distinguishing marker between these two cell types. CyTOF analysis helped define a unique OM expression profile whereby OM expressed simultaneously CD86 and CD206 which are known M1 and M2 macrophage markers, respectively. CyTOF also revealed that interactions with osteoblast and megakaryocytes upregulated the expression of many markers on OM including Embigin, Mac2, Stat3 and CD166. *In vitro* studies established that the CD166+ fraction of OM was required for maintaining hematopoietic activity. These results demonstrate that OM support HSC function and illustrate that megakaryocytes significantly augment the synergistic activity of osteoblasts and OM. We are currently merging our single cell RNA sequencing and CyTOF data to understand the mechanism through which OM support stem cell function and define possible mediators that are elaborated by OM to augment HSC function. At present, we have successfully substituted OM with recombinant CD166 and Lcn2 to support hematopoietic function *in vitro.* Future research will focus on further dissecting OM-osteoblast-megakaryocyte interactions. These data establish, for the first time, that the crosstalk between OM, osteoblasts and megakaryocytes is a novel network supporting HSC function.

Basic Science ***Graduate Student***

POSTER #22

ATAC SEQUENCING UNCOVERS ESTRADIOL-INDUCED GLOBAL CHANGES IN CHROMATIN ACCESSIBILITY LINKED TO GENE REPRESSION

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Significant efforts have been paid to identify gene targets of estrogen signaling that drive estrogen receptor-positive breast cancer. Previous work has shown that estrogen signaling directly and indirectly regulates approximately 25% of the breast cancer transcriptome. However, estrogen-induced overall chromatin remodeling, which sets the basis for gene induction or repression, remains unclear. More in depth analysis of open chromatin versus closed chromatin will provide greater mechanistic insight into gene regulation in breast cancer.

To investigate the effects of estradiol (E2) signaling on chromatin architecture, we employed Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq), a high-throughput method to map chromatin accessibility via a hyperactive transposase that inserts sequencing tags into open regions. ATAC-seq was performed on MCF-7 cells, an estrogen receptor-positive breast cancer cell line, treated with E2 or vehicle control for 1 or 3 hours. Results from the ATAC-seq were compared to RNA-seq data of 3-hours E2-treated MCF-7 cells to determine which regions of E2-regulated open or closed chromatin coincide with increased or decreased respective gene expression. We observed greater chromatin accessibility changes in genes that are repressed by E2 compared to genes that are induced by E2.

To mechanistically dissect this unexpected finding, we performed motif enrichment analysis of chromatin regions affected by E2. This analysis showed expected enrichment of estrogen response element (ERE), and pioneer factor FOXA1 and GATA3 response elements in E2-inducible genes with enhanced chromatin accessibility after E2-treatment. Surprisingly, genes with chromatin accessibility changes but repressed by E2 were enriched for pioneer factors FOXA2 and FOXA3 as well as bMYB and TEAD. Thus, it appears that FOXA family members have distinct roles in E2-inducible and E2-repressed genes. This work demonstrates novel findings regarding E2-induced chromatin remodeling and provides grounds for future work to study fundamental relationships between chromatin accessibility and gene regulation in breast cancer under E2 and anti-estrogen treated conditions.

Basic Science ***Graduate Student***

POSTER #23

PROFILING THE IMPACT OF MISSENSE MUTATIONS ON THE PROTEOME

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Over 100,000 missense mutations have been associated with diseases, including cancers and neurological disorders, with an estimated 60% of disease-causing mutations affecting protein stability and/or protein-protein interactions (PPIs). While structural data is available for many of these mutations, structure alone is not sufficient to provide a full picture of the effects of missense mutations on protein function or impacts on PPIs. Furthermore, currently there is not a high-throughput manner to study how missense mutations in a single protein affect the proteome. To address this gap, we developed a tool coupling cellular thermal shift assay (CETSA) with quantitative high-resolution mass spectrometry (HR-MS) to study how missense mutations affect protein thermal stability on a global proteome level. We focused on the ubiquitin-proteasome system (UPS) in yeast for the sake of these studies. Considering the essential requirement for the proteasome in global protein turnover, we are interested in two primary research questions. First, how do missense mutations within individual protein complex subunits affect the thermal stability of proteasome assembly? Secondly, how do changes in factors in the UPS impact thermal stability of degradation targets and the proteins they interact with? Due to the essential nature of the proteasome, studies of its function and substrates can prove difficult. To overcome this, we used yeast as a model system in which missense mutations within proteasome subunits provide a temperature “inducible” loss of proteasome activity. Cell lysate from each mutant strain was subjected to melt curve analysis through exposure to six temperatures according to an adapted CETSA protocol (adapted from Jafari, et al., 2014) for thermal profiling. The resulting soluble protein was subjected to LysC/Trypsin digestion followed by tandem mass tag (TMT) labeling. We have generated melt curves with at least 2,000 yeast proteins per genotype using reporter ion intensities for each temperature. A confidence filter of $R^2 = 0.95$ for fit to the sigmoidal melt curve was applied and significant changes in the T_m of individual proteins were determined using a 95% or 99% confidence interval. Successful development of this method provides a high-throughput way to profile the global effects of missense mutations on protein function and to characterize resulting changes in PPI networks. The ability to globally profile these effects on the proteome will provide insight into disease mechanisms and identify potential therapeutic outcomes.

Basic Science ***Graduate Student***

POSTER #24

TARGETING SURVIVIN TO OVERCOME DOCETAXEL RESISTANCE IN PROSTATE CANCER

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Background: Despite therapeutic advancements, castration-resistant prostate cancer (CRPC) remains the second most common cause of cancer-related mortality in men. Docetaxel is the first cytotoxic agent to show modest improvements in overall survival rate in patients with CRPC. Unfortunately, over half of these patients do not respond to treatment and ultimately all develop resistance. The mechanism mediating docetaxel resistance remains unknown. However, survivin, an inhibitor of apoptosis (IAP) family member, and known mediator of chemo-resistance has been previously associated with docetaxel resistance, as inhibition of survivin expression sensitized prostate cancer cells to docetaxel in vitro, and a small molecule inhibitor targeting survivin expression led to a significant regression in prostate cancer xenograft tumors when given in combination with docetaxel. However, how survivin may mediate docetaxel resistance in prostate cancer remains unknown.

Methods and Results: In this study, we tested the hypothesis that overexpression of nuclear survivin contributes to docetaxel resistance in prostate cancer cells. First, utilizing western blot to assess protein level and methylene blue assays to determine ability to proliferate under cytotoxic conditions, we found that survivin expression and docetaxel IC50 correlates strongly in five prostate cancer cell lines. Furthermore, using paired parental drug sensitive and stepwise selected docetaxel resistant cell lines, we determined that resistant cells overexpress survivin as compared to parental cells, and overexpression of survivin increases resistance to docetaxel. Stable overexpression of survivin increases prostate cancer cells resistance to docetaxel while transient siRNA knockdown of survivin decreases resistance to docetaxel. A small molecule inhibitor of survivin dimerization reduces survivin protein level and inhibits prostate cancer cell growth in vitro.

Future Directions: Our studies suggest that survivin is likely implicated in CRPC and treatment with a direct survivin inhibitor may sensitize resistant cells to docetaxel. We are in the process of determining how this IAP may mechanistically mediate docetaxel resistance.

Basic Science ***Graduate Student***

POSTER #25

MODULAR NANOPARTICLE SCAFFOLDS FOR TUNABLE DRUG DELIVERY

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Statement of Purpose: Glioblastoma accounts for a large percentage of all primary brain tumors. Conventional treatments of this aggressive cancer are currently ineffective and potentially damaging in an area with a low capacity for healing. The blood brain barrier offers extra difficulty to navigate. To this end, we have devised a scaffold that has the potential to cross the blood brain barrier and target cancer cells for tunable delivery. Specifically, we designed a nanoparticle (NP) system composed of poly-L-lysine (PLL) and modified heparin (Hep). Uniquely, we synthesized and incorporated heparin-methyltetrazine (Hep-mTz) to layered NPs for subsequent bio-orthogonal labeling of NPs with other norbornene-tagged bioactive cues for targeted delivery.

Methods: NPs were synthesized via sonicating PLL and Hep for 5 min (sonication conditions: 10% amplitude, 20kHz. Bronson Digital Sonifier). The weight ratio of Hep to PLL was systematically varied to obtain NPs with different surface charges. NP size and zeta potential were measured via dynamic light scattering. To improve the quality of the NPs, a two-step NP synthesis method was developed. Briefly, core NPs were synthesized using a 0.7 Hep/PLL weight ratio (0.7x). Additional Hep and/or Hep-mTz were added to the core NP solution, followed by secondary sonication. Hep-mTz was synthesized by reacting Hep with methyltetrazine-PEG-amine via standard carbodiimide chemistry. Hep-mTz was modularly added during the second step of NP preparation. For hydrogel crosslinking, desired amounts of 8-arm poly(ethylene glycol)-norbornene and NPs were mixed and incubated at 37°C for 24 hrs, followed by addition of dithiothreitol and LAP. The precursor mixtures were exposed to 365nm light (5 mW/cm^2) for 2 min for gel crosslinking and NP incorporation. Gel moduli were characterized by oscillatory rheometry.

Results: We designed a unique two-step sonication protocol to improve the tunability and quality of the polyelectrolyte NPs. The zeta potential could be tuned via controlling [Hep]/[PLL] ratio. The sonication method yielded NPs that were highly stable regardless of surface charge or size. From the DLS results, we decided to use [Hep]/[PLL] ratios of 0.7x and 1.3x for the subsequent tests. These NPs all had similar size distribution (~120nm) andwere stable for at least 1 month, which is ideal for extending therapeutics' shelf-life. With the core NP already formed after the first step sonication, a layer of Hep and/or Hep-mTz could be added to the NP surface rather than distributing throughout the entire NP. Modular tuning of Hep/Hep-mTz in the second sonication step yielded modular and tunable incorporation of ‘clickable’ motif (i.e., mTz) to NP surface. We tested if mTz incorporation could improve NP retention within the thiol-norbornene hydrogels. DMMB testing of the hydrogels provided evidence for an increase NP incorporation within the hydrogel, suggesting the Hep-mTz functionalized NPs were covalently ‘clicked’ into the gel network.

Basic Science ***Graduate Student***

POSTER #26

CXCL5 IS A MASTER REGULATOR OF THE DORMANCY SWITCH TO ACTIVATE METASTATIC COLONIZATION OF DORMANT BREAST CANCER CELLS DURING BONE METASTASIS

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Bone is one of the most common and most dangerous sites for metastatic tumor growth across cancer types, including breast cancer. At death, roughly 73% of women with breast cancer have bone metastasis, most often growing in highly vascularized bones. These metastases are detrimental to the patient's quality of life resulting in severe pain and immobility. However, bone metastasis is not considered curable with current therapies.

Circulating tumor cells sometimes become arrested in blood vessels or within tissue, remaining quiescent ('dormant) until the right conditions induce the cancer cells to grow in the metastatic site ('colonization'). Switching cancer cells from dormancy to colonization is rate-limiting for bone metastasis, sometimes taking decades to induce metastatic tumor growth. With few experimental models available to study this last step of metastasis, the switch from dormancy to colonization, or dormancy switch, has become one of the greatest challenges in cancer research. Most of the experimental models that are used today, fail to address this rate-limiting step in cancer progression.

Using our culture system, we then identified distinct culture conditions for tumor cell dormancy and colonization in bone. In fact, conditioned media from our dormancy culture conditions induced dormancy of cancer cells. Profiling of a panel of cytokines, chemokines, and growth factors identified the chemokine CXCL5 as a candidate to induce the switch from dormancy to colonization. Interestingly, bones from mice that harbored tumor cells before collection support a higher cancer cell proliferation rate in co-culture and secrete more CXCL5 than bones from healthy mice. This suggests that bones primed with cancer cells form a niche that actively supports the proliferation of metastatic cells and is inducible by CXCL5. In culture, devitalized bones and conditioned media from devitalized cultures support cancer cell proliferation, which suggests that the bone and/or marrow express an inhibitor of cancer cell proliferation. Additional studies using CXCL5 recombinant protein further suggest that CXCL5 is sufficient to overcome breast cancer dormancy and to promote proliferation in metastatic breast cancer. Together, this study supports the importance of the communication between the bone microenvironment and cancer cells to further promote metastatic colonization.

Basic Science ***Graduate Student***

POSTER #27

DELETION OF TUMOR DERIVED IL-6 MITIGATES METABOLIC ALTERATIONS ASSOCIATED WITH FAT AND MUSCLE LOSS IN MURINE PANCREATIC CANCER MODEL

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The profound loss of loss of fat and muscle known as cachexia is a devastating consequence of pancreatic ductal adenocarcinoma (PDAC). Cachexia contributes to reduced response and early termination of therapy, reduced quality of life and increased mortality. Tumor-derived factors lead to severe metabolic derangements causing negative energy balance and impaired protein turnover. The inflammatory cyctokine, interleukin-6 (IL-6) has been shown to be one of the critical factors in the development of cachexia in PDAC. IL-6 levels are consistently shown to be elevated in patients with pancreatic cancer and positively correlate with cachexia severity and mortality. To better understand the role of tumor-derived IL-6 in skeletal muscle metabolism and atrophy, we utilized the murine genetic PDAC model LSL-**K**rasG12D:LSL-Tr**p**53R172H:Pdx1-**C**re (KPC). Isolated KPC cells were deleted of IL-6 using CRISPR/Cas9 targeted mutagenesis. KPC and KPC IL-6$^{-/-}$cells were then orthotopically injected into wild type C57BL6/J mice to induce tumor formation.

The metabolic effects of the KPC and KPC IL-6 $^{-/-}$ tumors were evaluated using an integrated analysis of metabolomics and RNA-sequencing data. Comprehensive metabolic profiling utilized NMR and mass spectrometry based analyses of both serum and muscle tissue. RNA-sequencing analysis was carried out on muscle tissue extracts. The results of these analyses revealed that a number of critical metabolic alterations induced by the KPC tumor were either partially or completely mitigated by deletion of IL-6 in the tumor cells. The KPC model was characterized by an increased reliance on glycolytic metabolism as shown by an increase in circulating lactate and a 2.8 fold increase in the expression of pyruvate dehydrogenase kinase 4 (*Pdk4*), which blocks the entry of glucose derived pyruvate into the TCA cycle for oxidative metabolism. Lactate levels and*Pdk4*expression were unchanged versus control levels in the KPC IL-6 $^{-/-}$ model. Branched chain amino acids (BCAAs) were elevated in both the serum and muscle of KPC tumor mice and unchanged in KPC IL-6 $^{-/-}$ tumor mice. Expression of muscle branched chain keto acid dehydrogenase (*Bckdha*) was increased 2.1 fold in the KPC tumor mice. This is consistent with increased amino acid oxidation and the observed loss of skeletal muscle in KPC mice. KPC tumor mice had increased serum and muscle long chain acylcarnitines, suggestive of incomplete fatty acid oxidation (FAO), which was not observed in the KPC IL-6 $^{-/-}$tumor mice. Furthermore, Acetate levels were reduced in the KPC tumor mice along with a 6.5 fold increase in the expression of acetyl-CoA synthetase 1 (*Acss1*). This suggests that additional energy is being produced from diet/microbiome derived acetate. Taken together, our results suggest an important role for PDAC tumor-derived IL-6 in increasing the severity of cachexia by altering systemic tissue metabolism.

Basic Science ***Graduate Student***

POSTER #28

DELETION OF TUMOR-DERIVED IL-6 MAINTAINS MUSCLE MASS AND ATTENUATES LIPOLYSIS WITH EVIDENCE FOR SOLUBLE IL-6RA AS A DRIVER OF PANCREATIC CANCER CACHEXIA

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Cachexia, or weight loss in cancer, significantly increases morbidity and mortality and >85% of patients with pancreatic ductal adenocarcinoma (PDAC) will suffer from and die of cachexia. Interleukin-6 (IL-6) is increased in the blood of patients with PDAC and correlates with weight loss and cachexia severity. IL-6 has been well documented and has been shown to directly induce muscle and fat wasting. IL-6 activates signal transduction by first binding with the IL-6ra via classical (membrane bound receptor) or trans-signaling (soluble receptor) and then the IL-6/IL-6ra complex binds to the ubiquitously expressed glycoprotein 130 (gp130) on cell membranes. Importantly, previous studies suggest IL-6 trans-signaling promotes systemic inflammation in some diseases. This study aims to investigate the effects of tumor-derived IL-6 on cachexia and soluble IL-6ra expression in plasma, fat, and skeletal muscle of mice with PDAC.

We utilized a murine model of PDAC cachexia using orthotopic injection of tumor cells isolated from the genetic PDAC model LSL-**K**rasG12D:LSL-Tr**p**53R172H:Pdx1-**C**re (KPC). To determine the role of tumor-cell-derived IL6 in PDAC cachexia, the *Il6*gene in KPC cells was mutated using CRISPR/Cas9 to induce loss of expression.

Results showed animals bearing KPC tumors had higher mean plasma IL-6 versus KPC IL-6$^{-/-}$tumor and no tumor (WT) mice (291,159, 2 pg/mL respectively; $p<0.05$). Both KPC and KPC IL-6$^{-/-}$tumor mice had significant losses of epididymal fat mass versus WT animals (-81% and -37% respectively; $p<0.002$) however; KPC IL-6$^{-/-}$tumor mice had attenuation of fat loss versus KPC tumor mice. KPC tumor mice had increased loss of muscle mass (Quadriceps -21.2%, Tibialis -18.9%, Gastrocnemius -16.7%, Heart -26%; $p<0.05$) versus KPC IL-6$^{-/-}$tumor and WT mice. Analysis of IL-6ra levels in plasma showed significant increases in KPC tumor mice versus WT mice (12.69 vs 0.776 ng/mL; $p< 0.01$) and between KPC tumor vs KPC IL-6$^{-/-}$tumor mice (12.69 vs 1.68 ng/mL; $p< 0.01$). No differences in plasma IL-6ra was measured between KPC IL-6$^{-/-}$ tumor and WT mice. IL-6ra protein expression was increased in adipose tissue of KPC tumor mice versus KPC IL-6$^{-/-}$tumor and WT mice. IL-6ra protein expression was not different in the skeletal muscle between the groups. Interestingly, RNA sequencing analysis of fat and skeletal muscle showed a significant increase (+36.8-fold; p=5.14E-42) in IL-6ra reads in the skeletal muscle of KPC tumor mice versus the other groups while no difference in IL-6ra reads was observed in adipose tissue between groups. Finally, KPC tumor mice had decreased survival compared to KPC IL-6$^{-/-}$tumor mice (Median survival: 17 vs 24 days respectively; $p<0.002$). Taken together, these results suggest inhibiting tumor-derived IL-6 reduces circulating levels of IL-6 and IL-6ra, which is associated with increased survival and a reduction in fat wasting and cachexiogenic potential of the tumor.

Basic Science ***Graduate Student***

POSTER #29

AUTOCRINE FIBRONECTIN EXPRESSION INHIBITS BREAST CANCER METASTASIS

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The processes of the epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET) are linked to metastasis via their ability to increase invasiveness and enhance tumor-initiating capacity. Growth factors, cytokines and chemotherapies present in the tumor microenvironment are capable of inducing EMT, but the role of the extracellular matrix (ECM) in this process remains poorly understood. Using a novel tessellated three-dimensional (3D) polymer scaffolding we produce a fibrillar fibronectin matrix that is capable of inducing a EMT-like event that includes phosphorylation of STAT3 and requires expression of b1 integrin. Consistent with these findings, analysis of the METABRIC dataset strongly links high-level fibronectin (FN) expression to decreased patient survival. In contrast, *in vitro* analysis of the MCF-10A progression series indicated that intracellular FN expression was associated with non-metastatic cells. Therefore, we utilized differential bioluminescent imaging to track the metastasis of isogenic epithelial and mesenchymal cells within heterogeneous tumors. We demonstrate that mesenchymal tumor cells do not produce a FN matrix but instead retain FN within the cell and cannot complete the metastatic process, even when grown within a tumor containing epithelial cells. However, mesenchymal tumor cells form FN containing cellular fibrils capable of supporting the growth and migration of metastatic-competent tumor cells. Importantly, depletion of FN allows mesenchymal tumor cells to regain epithelial characteristics and initiate *in vivo* tumor grow within a metastatic microenvironment. In contrast to the tumor promoting functions of FN within the ECM our data suggest an intracrine function of FN in mesenchymal tumor cells that inhibits their metastatic potential.

Basic Science ***Graduate Student***

POSTER #30

BASAL SPLICING PROFILES PREDICT DOXORUBICIN RESPONSE IN CANCER CELL LINES

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Introduction:

Alternative splicing of gene transcripts is an important epigenetic control mechanism that increases the diversity of protein isoforms in a cell. Appropriate splicing is critical for differentiation and maintenance of cellular identity; however, aberrant splicing is known to contribute to development and progression of cancer. While certain protein isoforms have been found to help cancer cells evade chemotherapeutics, the impact of cellular splicing profiles on cancer drug response remains largely unexplored. In this study, we investigate the relationship between preexisting splicing alterations and response to the chemotherapeutic doxorubicin. We demonstrate the predictive power splicing information can lend to drug response models and report differences in splicing profiles between doxorubicin sensitive and resistant cancer cell lines. We introduce a new method for group analysis of splicing data using the quasi-binomial distribution that is simple, powerful and well suited for comparison of large numbers of samples. Finally, we perform RNA-binding protein motif enrichment at differentially spliced exons to characterize proteins that may play a role in their regulation.

Methods:

Area-under-the-concentration-response-curve (AUC) values from doxorubicin profiling data in the Cancer Therapeutic Response Portal were matched to RNAseq files corresponding to the same cell lines from the Cancer Cell Line Encyclopedia. Using the upper and lower tertiles of the AUC value distribution to classify cells, we obtained a total of 253 sensitive and 258 resistant cell lines. RNAseq data was then analyzed with Mixture of Isoforms (MISO) using exon-centric splicing quantification. Expression analysis was conducted using edgeR. Machine learning was performed using ElasticNet logistic regression. Data was divided into 70% training and 30% testing sets, and a 10-fold cross validation was used to train predictive models.

Differential splicing analysis was performed using Generalized Linear Modeling (GLM) with quasi-binomial distribution. Splice junction read counts for skipped-exon events and group classes were used to fit the quasi-binomial GLM. Results were filtered by group weight significance at an FDR of <=0.01 and >=10% difference in mean exon inclusion ratio. Gene symbols for events were submitted to WebGestalt biological processes overrepresentation analysis. RNA-binding protein (RBP) motif analysis was performed on regions surrounding and including skipped exons using FIMO.

Results:

Three predictive models were built with ElasticNet logistic regression; Splicing-only, expression-only and a combined “splicing+expression” model. The splicing-only model produced a sensitivity of 0.70, specificity of 0.86, accuracy of 0.78, ROC area-under-the-curve of 0.84 and Pvalue of 3.9e-12. The expression-only model

had the highest sensitivity (0.83), while the combined model had the highest specificity (0.88), accuracy (0.83), ROC area-under-the-curve (0.90) and Pvalue (2.8e-16). We identified 277 differentially spliced events between doxorubicin sensitive and resistant groups. Significantly enriched biological processes supporting the role of alternative splicing in epithelial-mesenchymal transition (EMT) and cellular differentiation were found. RBP motif analysis showed two RBP's, ELAVL1 and RBFOX1, were enriched in differentially spliced events and also differentially expressed. These proteins have also been associated with EMT and other cancer related processes.

Conclusions:

- Splicing information can be used to predict drug response

- Adding splicing information to expression-based models can improve model performance

- Biological process and RBP analysis confirms previous studies linking splicing to EMT and cellular identity

- Splicing may contribute to EMT in tumor sub-populations, helping to drive cellular plasticity and stemness

- More research is needed to understand the role of splicing in drug response

Basic Science ***Graduate Student***

POSTER #31

DETERMINING DNA DAMAGE RESPONSE PROTEINS ASSOCIATED WITH OVARIAN CANCER STEM CELL RESISTANCE TO PLATINUM-BASED CHEMOTHERAPY AGENTS

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A majority of ovarian cancer is detected at a late stage and patients with Stage 4 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. 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 known to be enriched in disease relapses and responsible for platinum chemo resistance. We hypothesize that OCSCs undergoes a DDR that is distinct from non-OCSCs, which provides them with survival advantage when treated with chemotherapy, and hence cause cancer reoccurrence.

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. Although *BRCA1* mutation is common in ovarian cancer, less than 10% of high grade serous ovarian cancer, the most aggressive type of ovarian cancer, have *BRCA1* mutation. Our preliminary results using cells from a xenograft mouse study showed decrease in *BRCA1* RNA levels in residual OCSCs that survive after treatment with Pt- based drugs. Additionally, we observed decreased levels of BRCA1 protein levels after acute treatment with Pt-based drugs in vitro. Thus, we hypothesize that decrease in BRCA1 protein levels promote cell survival in response to treatment with Pt-based chemotherapeutic drugs. Our current focus is to determine how BRCA1 protein is regulated in OCSCs. Our overall goal is to determine other potential proteins or pathways that can be targeted to sensitize OCSCs to Pt-based chemotherapy.

Basic Science ***Graduate Student***

POSTER #32

BMI1 LOCALIZATION TO SITES OF DNA DAMAGE AS A POTENTIAL MECHANISM FOR DEVELOPMENT OF CISPLATIN RESISTANCE

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Platinum agents are the most commonly used class of chemotherapeutic drugs for treatment of ovarian cancer patients. The platinum agents, cisplatin and carboplatin damage DNA by forming adducts with adjacent guanines, a high density of which is found in the promoter CpG islands of genes. Resistance to cisplatin and other platinum drugs is the major cause of mortality among ovarian cancer patients. Recent evidences have shown that aberrant DNA hypermethylation of genes and their subsequent transcriptional repression plays a role in development of cisplatin resistance in ovarian cancer. However, the mechanism of initiation of this aberrant DNA methylation and consequent transcriptional repression is not known. We, along with other groups, have demonstrated that transient transcriptional repression occurs in the vicinity of sites of enzyme induced double strand breaks, to promote repair. Our group has also demonstrated that after completion of the repair, transcription resumes in most of the cells. However, in a small percentage of cells, repressive proteins are retained at some loci causing persistent transcriptional repression and gene silencing. BMI1, a member of Polycomb repressive complex 1, has been shown to localize to sites of ionizing radiation - induced double strand breaks and ubiquitinate H2A at the damage sites. During development and cell differentiation, ubiquitination of H2A at K119 by BMI1 is known to be involved in gene silencing. BMI1 is also overexpressed in cisplatin resistant cells. We hypothesize that BMI1 ubiquitinates H2AX at K119 at sites of cisplatin-induced DNA damage and contributes to transcriptional repression. Our preliminary data demonstrates H2AX ubiquitination and BMI1 co-localization with the damage marker ¿H2AX after cisplatin treatment. A portion of this ubiquitination is dependent on ATM and nucleotide excision repair pathway proteins- XPA and CSB suggesting that these repair proteins play a role in recruiting BMI1 to sites of damage where it ubiquitinates H2AX at K119 contributing to transcriptional repression. This recruitment may occasionally cause stable gene silencing ultimately contributing to the development of cisplatin resistance. Importantly, we demonstrate that candidate genes that are known to be silenced by DNA methylation in cisplatin resistant cells, are transcriptionally repressed following acute cisplatin treatment. We plan to determine if BMI1 is responsible for reduced expression of these genes after acute treatment and their silencing in cisplatin resistant cells. Understanding the role of BMI1 in platinum resistance will enable us to design therapies to avert the development of drug resistant ovarian cancers.

Basic Science ***Graduate Student***

POSTER #33

ERK1/2 PHOSPHORYLATION OF THE ONCOGENIC TRANSCRIPTION FACTOR ERG RELIEVES TRANSCRIPTIONAL REPRESSION BY THE PRC2

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The *TMPRSS2-ERG* chromosomal rearrangement occurs in ~50% of prostate cancers and results in androgen mediated aberrant expression of the transcription factor ERG. Upon expression in the prostate, ERG acts as an oncogene and promotes cell migration, invasion, angiogenesis, and epithelial to mesenchymal transition. Active Ras/MAPK signaling has previously been demonstrated to be necessary for ERG's transcriptional activation and for ERG mediated oncogenic phenotypes, however, the exact mechanism of this activation was not known. Recently, our group has demonstrated that ERK1/2 directly phosphorylates ERG first at S215, which facilitates a conformational change and subsequent phosphorylation of ERG at S96. Phosphorylation at S96 abrogates ERGs interaction with EZH2 and the Polycomb Repressive Complex 2 (PRC2) and is necessary for transcription of ERG target genes.

The PRC2 has previously been demonstrated to repress transcription of target genes via multiple mechanisms. In its canonical role the PRC2 catalyzes the tri-methylation of H3K27 which results in the formation of heterochromatin, however, the PRC2 has also been demonstrated to directly methylate transcription factors to repress their activation. In order to determine the mechanism by which the PRC2 represses ERG transcriptional activation we have explored both of these mechanisms. First, we have conducted ChIP-Seq of H3K27me3 in cell lines expressing phospho-mutants of ERG at S96 to compare the distribution of the modification dependent on ERGs differential interaction with the PRC2. In parallel, we also sought to determine if the PRC2 could directly methylate ERG to repress its transcriptional activity. We performed Mass Spectrometry post-translational modification analysis of purified ERG incubated with purified PRC2 as well as ERG immune-precipitated from cells to determine potential methylation sites. Overall, this study provides mechanistic insights as to how an oncogenic transcription factor's activity is repressed and how signaling pathways act to relieve this repression.

Basic Science ***Graduate Student***

POSTER #34

EN ROUTE TO THE LYMPH NODE: LYMPH CIRCULATING TUMOR CELLS ARE PHENOTYPICALLY DIFFERENT FROM TUMOR CELLS CIRCULATING IN THE BLOOD

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In breast cancer, the spread of tumor cells from the primary tumor to the regional lymph node denote bad prognosis and patients with lymph node metastases have worse outcome. Breast cancer patients with no tumor cells in their lymph nodes, stage I, has 95.9% 10-year survival rate compared with patients with tumor cells spread to their lymph nodes (defined as stage III), the 10-year survival is less than 40%. The association between lymph node and distance metastasis was the basis for TNM staging and sentinel lymph node surgical resection in the hope of improving survival. However, numerous clinical trials have shown that lymph node removal does not always improve patient survival. In this work, we examined the relationship between primary tumor, lymph node metastasis and tumor cells circulating in the lymphatic and vascular systems. We used a microsurgical technique to collect draining lymph *in situ* from a growing tumor prior to its entry into the nearest draining lymph node in syngeneic animals and patients with breast cancer. Also, we have collected the primary tumor, lymph node metastasis and circulating tumor cells (CTC) in the blood circulation. Gene and protein expression patterns of the primary tumor cells are so similar to the lymph node metastasis but both are different from that of tumor cells circulating in the lymph (LCTC) or the blood (BCTC). Unlike BCTC, LCTCs are found in clusters that exhibited a hybrid E/M phenotype, displayed a CSC $CD44^{hi}/CD24^{hi}/ALDHA1^{hi}$ signature, grow as mammospheres and are highly tumorigenic. We also showed that the lymph is enriched in tumor-derived exosome proteins and EGF growth factor. Preliminary findingshint to that cells circulating in the lymphatic and cells circulating in the blood use different route of spread –aided by the tumor-secreted factors such as chemokines, cytokines and growth factor

Basic Science ***Graduate Student***

POSTER #35

MUTANT AND WILD-TYPE P53 FORM COMPLEXES WITH P73 UPON PHOSPHORYLATION BY THE KINASE JNK

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The transcription factors p53 and p73 are critical to the induction of apoptotic cell death, particularly in response to cell stress that activates c-Jun N-terminal kinase (JNK). Mutations in the DNA-binding domain of p53, which are commonly seen in cancers, result in conformational changes that enable p53 to interact with and inhibit p73, thereby suppressing apoptosis. In contrast, wild-type p53 reportedly does not interact with p73. We found that JNK-mediated phosphorylation of Thr^{81} in the proline-rich domain (PRD) of p53 enabled wild-type p53, as well as mutant p53, to form a complex with p73. Structural algorithms predicted that phosphorylation of Thr^{81} exposes the DNA-binding domain in p53 to enable its binding to p73. The dimerization of wild-type p53 with p73 facilitated the expression of apoptotic target genes [such as those encoding p53–up-regulated modulator of apoptosis (PUMA) and Bcl-2-associated X protein (BAX)] and, subsequently, the induction of apoptosis in response to JNK activation by cell stress in various cells. Thus, JNK phosphorylation of mutant and wild-type p53 promotes the formation of a p53/p73 complex that determines cell fate: apoptosis in the context of wild-type p53 or cell survival in the context of the mutant. These findings refine our current understanding of both the mechanistic links between p53 and p73 and the functional role for Thr^{81} phosphorylation.

Basic Science ***Graduate Student***

POSTER #36

TARGETING DAB2IP IN OVARIAN CANCER STEM CELLS

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Ovarian cancer is a leading cause of death from gynecologic malignancy. The majority of women diagnosed with advanced-stage epithelial ovarian cancer experience lethal tumor relapse after initial therapy. Failure to kill ovarian cancer stem cells (OCSCs) by the end of conventional chemotherapy is considered as a main reason for tumor relapse and metastasis, accompanied by chemoresistance. DAB2IP, a novel member of Ras GTPase-activating (GAP) protein family, has been shown to inhibit cancer progression and downregulated in many different types of human cancers, including high-grade serous ovarian cancer (OC). Furthermore, reduced expression of DAB2IP significantly correlated with poor survival rate in patients with advanced OC, and loss of DAB2IP in prostate and colon cancer promoted CSC- like features, suggesting a critical role for DAB2IP in modulating CSCs survival. Based on a possible role in regulating OCSCs, we hypothesized that targeting DAB2IP using epigenetic therapy will prevent tumor relapse and metastasis. The goal of the current study was to investigate the tumor suppressor role of DAB2IP in OC in the context of OCSCs and explore underlying signaling pathways mediated by DAB2IP. OCSCs and non-CSCs subpopulations were isolated from OC cell lines Kuramochi, OVCAR3 and COV362 based on aldehyde dehydrogenase (ALDH) activity. ALDH (+) cells, isolated by FACS, are known to initiate tumors and meet all CSC criteria, while ALDH (-) are considered to be non-CSC. Expression of DAB2IP mRNA and protein was examined using qPCR and western blot. DAB2IP expression in ALDH (+) cells was lower ($P<0.05$) compared to ALDH (-) cells. Enforced overexpression of DAB2IP decreased ($P<0.05$) the OCSC population, and inhibited stemness-related phenotypes, including reduced spheroid formation ability after 14-day incubation under stem cell conditions and decreased colony formation. Furthermore, elevated DAB2IP expression decreased ($P<0.05$) cisplatin IC50 (15.63mM vs 8.27mM) and cell migration capacity in transwell assays, suggesting DAB2IP plays a role in regulating OCSC function. Transcriptome analysis of DAB2IP overexpression cells and control cells was carried out using RNA-sequencing and bioinformatics analysis. RNA-sequencing data revealed that DAB2IP overexpression significantly (FDR < 0.05, fold change > 2) altered expression of 449 genes, including important stem cell markers *(ALDH1A1, LGR5, PROM1, TWIST1)* and ATP-binding cassette transporters *(ABCB11, ABCG1, ABCA2, ABCA3, ABCA5)*. These data support a key role for DAB2IP in modulating CSC-driven ovarian cancer progression. We suggest that a better understanding of DAB2IP will facilitate development of therapeutics targeting OCSCs to impact tumor relapse and chemoresistance in ovarian and perhaps other solid tumors.

Basic Science ***Graduate Student***

POSTER #37

INVESTIGATING THE ROLE OF WILD TYPE TFE3 IN RENAL CELL CARCINOMA CELLS HARBORING TFE3 FUSIONS WITH SPLICEOSOME MACHINERY ASSOCIATED GENES

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Background: Translocation Renal Cell Carcinoma (tRCC) represents an aggressive subtype of kidney cancer associated with various gene fusions involving translocation of one of two members of micropthalmia transcription factor (MiT) family, TFE3 or TFEB. Despite the identification of multiple TFE3 gene fusions in tRCC, heterogeneous phenotype and various dysregulated signaling pathways resulting from variety of gene fusion partners pose a challenge to establish effective treatments for these patients. In this work, we assessed the role of wild type TFE3 (TFE3-WT) in RCC cell bearing TFE3 fusion with genes associated with pre-mRNA splicing factor machinery (NONO, SFPQ and PRCC).

Methods: Endogenous SFPQ-TFE3 expressing cells, RP-RO7, were generated from patient derived xenograft established in NSG mice. UOK-109 and UOK-146 (kindly provided by Dr. Marston Lenehan, NCI) were used as endogenous NONO-TFE3 and PRCC-TFE3 expressing cells. RNA interference (RNAi) mediated knockdown with differential exon targeting strategy was employed to study TFE3-WT loss of function in RP-R07, UOK-109 and UOK-146, respectively. Real time monitoring of cell viability assay with multiplex readout were developed to investigate the effect of TFE3-WT loss of function in 2D and 3D culture system. Two different 3D models were used;1) Tumor growth model, in which cancer cell interaction with extracellular matrix represented in a scaffold aided spheroid system; 2) Invasion model, in which cell ability to invade basement membrane barrier was modeled with matrix restricted spheroid, and the invasion trajectories were observed and quantified. Exogenous expression of TFE3-WT-EGFP was utilized to study the protein subcellular localization in RP-R07, UOK-109 and UOK-146 2D cultures system.

Results: Consistent with the expression of chimeric TFE3, TFE3-WT-EGFP ectopic expression demonstrated strong nuclear localization in RP-R07, UOK-109 and UOK-146. Multiplex readout of cells viability assay showed that transient loss of TFE3-WT in RP-R07, UOK-109 and UOK-146 curtails the proliferation rate of these cells in 2D and 3D models in fusion partner dependent manner. 3D invasion assay coupled with RNAi strategy in RP-R07, UOK-109 and UOK-146 demonstrated the role TFE3 in invasion potential of these cells in fusion partner dependent fashion. Downstream analysis suggested that the TFE3-WT transient silencing affected cell proliferation and invasion ability via inhibition of the IRS-PI3K-mTOR axis.

Conclusions: Our results indicate that TFE3-WT plays a common role in the biology of tRCC irrespective of TFE3 fusion partner. TFE3-WT loss of function in tRCC resulted in inhibition of cell proliferation, spheroid growth and invasion ability. Our results also indicate a coregulatory role between TFE3-WT and TFE3 fusion protein.

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

POSTER #38

TIME SERIES TRANSCRIPTOME AND FLOW CYTOMETRIC ANALYSIS OF CULTURED HUMAN CELL LINES: IMPLICATIONS FOR GENOMIC INTEGRITY IN RESPONSE TO PROTEASOME INHIBITION

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The eukaryotic somatic cell cycle is divided into four phases: mitosis (M), first gap (G1), DNA synthesis (S), and second gap (G2). Phase transitions are characterized by irreversible and switch-like signaling dynamics primarily governed by the post-translational modification and rapid destruction of periodically expressed gene products. Exit from mitosis is controlled by the anaphase-promoting complex (APC), which ubiquitylates and targets M and S phase regulators for degradation by the proteasome. The APC cofactor Cdh1 is primarily credited for managing the cell's transition through mitosis, though it also remains active throughout G1 to suppress continued translation of mitotic cyclins. It has been reported by our laboratory that ablation of the APC cofactor Cdh1 results in both premature and prolonged DNA replications, suggesting that it more generally participates in the precise dynamics of genome replication by the dividing cell. To further characterize this, we exposed three human cell lines to the proteasome inhibitor MG132 and interrogated the G1 protein levels of several mitotic regulators using flow cytometry. Using varying concentrations of MG132 in combination with transcriptional (actinomycin D) and translational (cycloheximide) inhibitors, we demonstrated that translation of these proteins occurs due to remnant transcript carried over from mitosis. Furthermore, using synchronized cell cultures we showed that sub-optimal concentrations of MG132 permit cells to exit mitosis, accumulate mitotic regulators in G1, and progress into another round of DNA replication. To identify other genes that may be aberrantly expressed in G1 by compromised proteasome activity, we devised a flow cytometric method to specifically sort G1 from mitotic cells and obtain sufficient high-quality RNA for next-generation sequencing without pre-amplification. We identified cell cycle-regulated genes and established their profiles by hierarchical clustering using triplicate data from six hour time series of synchronized human cells (HeLa, RPE-1, U2OS). We then used a group-based prediction system algorithm (GPS-ARM 1.0) to identify Cdh1 recognition motifs in the human UniProt library and cross-referenced this list with published proteomic databases to identify genes in our dataset that encode known and putative targets of APC-mediated proteolysis. Functional annotation revealed significantly enriched pathways related to mitotic progression, DNA repair, and DNA synthesis.

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

POSTER #39

KINASE ACTIVITY OF FIBROBLAST GROWTH FACTOR RECEPTORS REGULATE ACTIVITY OF THE PAPILLOMAVIRUS E2 PROTEIN

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The papillomavirus (PV) is a double-stranded DNA tumor virus infecting cervix, mouth, and throat tissues causing cervical, anal, and oropharyngeal *cancers.* Understanding the mechanisms of the replicative life cycle of the virus may bring to light direct targets and treatments against viral infection. The PV genome replication program involves three stages: initial amplification from a single to a few copies per cell, a cell cycle synchronized maintenance phase, and a keratinocyte differentiation dependent late stage where the genome is amplified to thousands of copies. The viral protein E2 is responsible for the replication of the virus. We recently found that the several members of the fibroblast growth factor receptors (FGFR1-4) interact with and phosphorylate the PV E2 protein. We further characterized the effect of this interaction with FGFR2 and FGFR3, which are both highly expressed in skin tissue, with high risk HPV-16 and HPV-31. Interaction of these tyrosine kinases with PV E2 is nuclear and mediated through the E2 transactivation domain. Activation of FGFR2 and FGFR3 suppressed viral replication and FGFR3 depletion in cell lines that maintain HPV-31 episomes increased viral copy number. Our next goal was to determine the direct tyrosine(s) target within the PV E2 induced by FGFR2 and FGFR3. Mutational analysis of the conserved tyrosine (Y) 138 in PV E2 to phenylalanine (F) did not display reduced FGFR3-induced replication as seen for wild-type E2; in contrast FGFR2 still suppressed viral replication through Y138F. These data suggest that Y138 may be a direct target for FGFR3. We propose that FGFR phosphorylates E2, which restricts PV replication and prevents lytic infection.

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

POSTER #40

CONCURRENT MUTATIONS IN KRISTEN RAT SARCOMA AND AT-RICH INTERACTIVE DOMAIN 1A ARE SUFFICIENT TO CAUSE THE DEVELOPMENT OF SPONTANEOUS ENDOMETRIOSIS AND CANCERS IN MICE

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Endometriosis is a debilitating gynecologic disease that effects 5-10% of US women. Women with endometriosis are at increased risk of ovarian cancer, specifically, endometrioid and clear cell ovarian adenocarcinomas. Recent work showed that loss-of-function mutations of AT-rich interactive domain 1A (*ARID1A*) were common in these endometriosis-associated ovarian cancers. However, work from us and others showed that deletion of *Arid1a* in mouse models was not sufficient to induce cancer. Because 29% of endometriosis-associated ovarian cancers from women have oncogenic mutations in Kristen Rat Sarcoma (*KRAS)*, we added oncogenic *Kras* to our *Arid1a* conditional knockout mouse model. To study the interactions between loss of *Arid1a* and gain of oncogenic *Kras*, we created and characterized the $Arid1a^{flox}/Arid1a^{flox};Kras^{lox\text{-}stop\text{-}lox\text{-}G12D\ (mut)}/Kras^{lox\text{-}stop\text{-}lox\text{-}G12D\ (+)};Amhr2^{Cre}/Amhr2^{+}$ (*AKC*) mouse. *Arid1a* knockdown was assessed by immunohistochemistry in the ovaries of various aged mice (12 weeks – 1 year) and quantitative polymerase chain reaction showed a reduction in mRNA at 1 year. When bred to wild-type males, *AKC* females mice demonstrated decreased number of pups per litter compared to controls (11 versus 1.4, p = 0.001, n=4-5 per group). Additionally, the ovaries of *AKC* mice are characterized by immature follicles and appear to improperly cycle as witnessed by decreased trending in progesterone (12.6 versus 4.1, p = 0.07, n=7 per group) and significantly elevated follicle stimulated hormone (5.3 versus 36.7, p = 0.001, n=7 per group) of 1 year mice. Long-term survival studies demonstrated that *AKC* female mice had decreased survival with 70% of mice dying by 335 days (n=20-21 per group, p = 0.01) compared to control mice, 365 days. Uterine to body weight ratios in *AKC* mice were decreased from 6 to 12 weeks, while ovarian weight to body weight ratios were increased starting as early as 3 weeks. Gross ovarian endometriosis-like lesions were observed as early as 4 weeks in *AKC* mice with over 59% (13/22)penetrance by 12 months. Detailed pathological evaluation of all *AKC* ovaries confirmed the presence of endometriosis with endometrial glands, stroma, and hemosiderin-laden macrophages. 64% (7/11) of *AKC* mice had pathology confirmed endometriosis by 9 months of age which increased to 73% (16/22) at 12 months. Interestingly, 27% (3/11) of *AKC* female mice at 9 months developed benign hemangiomas. 18% (4/22) of *AKC* female mice at 12 months developed malignant ovarian spindle cell sarcomas which were unlikely to be derived from the histologically normal uterus. Three 9-12 month *AKC* females had thickened but non-invasive papillary, papillary squamous proliferation, or cutaneous squamous cell carcinoma at the skin/vaginal junction. Five *AKC* females presented with lung lesions and two were pathologically confirmed as pulmonary adenocarcinoma. The *AKC* female mice represent a new and the only spontaneous developmental model of endometriosis currently available. This work was supported by NCIR03CA19127**.**

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

POSTER #41

NOVEL INTERACTION BETWEEN PROTEIN TYROSINE KINASE 2B (PTK2B/PYK2) AND HUMAN PAPILLOMAVIRUS E2

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The human papillomavirus (HPV) is a small, non-enveloped DNA virus that infect mucosal and cutaneous epithelia. High-risk HPV types cause approximately 5% of all cancers worldwide, with tumors of the cervix and oropharynx most common. There is no medical treatment for persistent HPV infections. The HPV E2 protein regulates several activities in the viral life cycle including viral gene expression, replication and mitotic partitioning of the viral genome. Apart from its interactions with other viral proteins, E2 interacts with numerous host proteins which influence its function as well as other cellular processes. Interactions of E2 with host tyrosine kinases like Fibroblast growth Receptors (FGFR) and subsequent phosphorylation have been shown to suppress E2-dependent viral replication. There are several conserved tyrosine residues in the high-risk HPV subtypes which are potential targets for phosphorylation and the host kinases acting on these still remain unknown.

PYK2 is a non-receptor tyrosine kinase which belongs to the family of focal adhesion kinases. PYK2 has been shown to be in complex with HPV 18 E2 in proteomics studies and there is evidence for expression of this kinase in skin. We hypothesized that PYK2 interacts with E2 and phosphorylates it to repress viral replication. Using co-immunoprecipitation experiments, we show that PYK2 physically interacts with HPV 31 and HPV 16 E2. Knockdown of PYK2 in keratinocytes that stably maintain HPV 31 (CIN612) or HPV 16 (W12) significantly increased viral replication. Treatment of these cell lines with a known PYK2 inhibitor PF-431396 also resulted in enhanced viral replication. We have made a series of non phosphorylatable (F) and phosphomimetic (E)mutants of HPV31 E2 to identify the tyrosine residue/s that are likely to be modified by PYK2 kinase. We are also employing a mass spectrometric approach to discover the post translational modifications on E2 when co-expressed with WT as well as kinase dead PYK2. Elucidation of mechanisms by which PYK2 regulates the HPV life cycle will provide possible targets for inhibition of viral replication.

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

POSTER #42

BREAST EPITHELIAL CELL LINES FROM NORMAL BREAST WITH LUMINAL AND INTRINSIC SUBTYPES –ENRICHED GENE EXPRESSION DOCUMENT INTER-INDIVIDUAL DIFFERENCES IN DIFFERENTIATION CASCADE

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Breast cancers are classified into five intrinsic subtypes based on comparative gene expression analyses between tumors. It is suggested that these intrinsic subtypes originate from specific subpopulation of epithelial cells within the path of stem-progenitor-mature cell hierarchy of the normal breast. However, normal breast epithelial cell line resources representing these intrinsic subtypes are yet to be created. Using normal breast tissues of ancestry-mapped Caucasian, African American, and Hispanic women and a primary cell culturing system that provides a replenishable source of normal epithelial cells of different lineages including estrogen receptor-positive mature luminal, luminal progenitors, and stem cells, we created 15 human telomerase-immortalized breast epithelial cell lines. RNA sequencing and PAM50 intrinsic subtype clustering algorithms were used to identify the intrinsic subtypes of the immortalized cell lines as well as to distinguish these cell lines from MCF10A and human mammary epithelial cell lines (HMECs), which are commonly used as "normal" breast epithelial cells in the literature. Unlike MCF10A and HMECs, which are enriched for basal-like gene expression pattern, our cell lines classified into luminal A, basal, and normal-like subtypes. This was also reflected in the immunofluorescence staining with KRT 14 and KRT 19. The cell lines were dual positive for KRT 14 and KRT 19, but in varying proportions. We have also created cell lines from CD201+/EpCAM- cells that are likely "normal" counter part of claudin-low subtype of breast cancers. Consistent with recent discoveries of genetic variations in gene regulatory regions among general population that contribute to widespread differences in gene expression under "normal" state, these cell lines showed inter-individual differences in stemness/differentiation capabilities and variable basal activity of signaling molecules such as NF-kB, AP-1 and pERK. These cells formed acini on a matrigel and ductal structures on 3-dimensional collagen or hydrogel. The majority of breast cancers are believed to originate from luminal progenitor cells, which are well represented our cell lines. Therefore, the resources developed in this study are ideal to delineate the impact of inter-individual and ethnic differences in normal breast biology on breast cancer initiation and progression as well as to determine whether cell-type-origin instead of genomic aberration drives intrinsic subtype-enriched gene expression patterns in breast tumors.

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

POSTER #43

CIRCULATING PLASMA-MIRNAS AS BIOMARKERS FOR CYP2B6 ACTIVITY IN VIVO

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BACKGROUND:

Plasma miRNA profiles have been associated with liver disease and function. Cell-free miRNAs that circulate in blood may serve as biomarkers of hepatic drug metabolism. We tested this possibility by correlating circulating miRNA expression with the in vivo activity of CYP2B6, an enzyme that metabolize clinically important drugs including chemotherapy drugs such as cyclophosphamide. CYP2B6 expression can be induced through nuclear receptors such as pregnane X receptor (PXR), constitutive androstane receptor (CAR) and glucocorticoid receptor (GR). The CYP2B6 gene is highly polymorphic and its activity shows wide inter-individual variability. However, substantial variability in CYP2B6 activity remains unexplained by the known CYP2B6 genetic variations. miRNAs can alter the expression of CYP2B6 mRNA or its upstream regulators and cause additional variability in CYP2B6 activity

METHODS:

CYP2B6 activity was determined using the disposition of efavirez as a probe drug. Healthy volunteers (n=72) received a single 600 mg oral dose of efavirenz. Blood samples (0.5-168 hours) and urine samples (0-48 hours) were collected after dosing. Concentrations of efavirenz and its metabolites in plasma and urine were measured using LC/MS/MS. DNA was genotyped for CYP2B6 variants using Taqman assays. miRNAs were isolated from baseline plasma samples and their expression was quantified using next generation sequencing. The samples were randomized into training (n=48) and testing datasets (n=24). A linear model that included the effect of CYP2B6 genotype and miRNA expression was developed, and was used to predict enzyme activity. The robustness of the model was tested by re-randomizations of the training/testing datasets. The average Pearson's correlation between predicted and observed Cmax ratios was adopted to assess the overall model performance.

RESULTS:

Expression of 2387 miRNAs were quantified out of which 7 miRNAs which, together with the CYP2B6-genotype, was shown to be predictive markers for CYP2B6 activity. Two of these miRNAs have predicted binding sites in the CYP2B6 gene. The average Pearson's correlation (R) between the predicted Cmax ratios using the linear model with 8 features (7 miRNA + genotype) and the observed values was 0.6. Thus, 36% (R^2) of the variability of *in vivo* CYP2B6 activity was explained using this model. This is a significant improvement over the model using only genotype, which explained only 6% of the variability of *in vivo* CYP2B6 activity.

CONCLUSION:

We have built a prediction model for *in vivo* CYP2B6 activity based on genotype and circulating plasma miRNAs. Using both miRNAs and genotype, the prediction significantly outperforms using genotype alone. Thus, we have demonstrated that circulating plasma miRNAs can be valuable biomarkers for CYP2B6 activity.

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

POSTER #44

HETEROZYGOUS DELETION OF CHROMOSOME 17P RENDERS PROSTATE CANCER VULNERABLE TO THE INHIBITION OF RNA POLYMERASE II

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Heterozygous deletion of human chromosome 17p (17p) is one of the most frequent genomic events that contribute to tumorigenesis. Beyond the tumor suppressor *TP53*, whose mutation has been long known as a primary tumor driver, the biological and clinical consequences of those co-deleted genes in the 17p region remains to be fully elucidated. Reduced doses of these linked passenger genes may create therapeutic vulnerability. The *POLR2A* gene, encoding the catalytic subunit of the RNA polymerase II (RNAP2), is included in a ~20-megabase deletion region of 17p in 63% of metastatic castration-resistant prostate cancer (CRPC). Inhibition of RNAP2 with a-amanitin-based antibody drug conjugates (ADCs) selectively inhibited the proliferation, survival and tumor growth of the CRPC cells with heterozygous deletion of 17p. Using a genome-wide CRISPR-Cas9 screen, we discovered that heterozygous loss of 17p confers a selective dependence on the ubiquitin E3 ligase Ring-Box 1 (RBX1). RBX1 activates POLR2A by the K63-linked ubiquitination and thus elevates the RNAP2-mediated mRNA synthesis. Depletion of RBX1 alone showed selective impairment of cell viability for the $17p^{loss}$ CRPC cells. Importantly, combined inhibition of RNAP2 and RBX1 profoundly suppressed the growth of CRPC in a synergistic manner, which potentiates the therapeutic effectivity of the a-amanitin-based ADCs. Given the limited therapeutic options for CRPC, our findings identified RBX1 as a potential vulnerability and drug target augmented by a common 17p deletion event in human CRPC.

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

POSTER #45

METABOLIC REPROGRAMMING BY DICHLOROACETATE MITIGATES MUSCLE WASTING IN CANCER CACHEXIA

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The severe muscle wasting disorder known as cachexia affects upwards of 80% of advanced cancer patients and for nearly 30% of patients this will be the ultimate cause of death. Cachectic patients experience tumor-driven metabolic reprogramming that leads to negative energy balance and impaired protein turnover. Recent studies from our laboratory have shown that cachexia is characterized by severe metabolic derangements in the skeletal muscle, including perturbations to glycolysis, fatty acid oxidation and amino acid catabolism. Dichloroacetate (DCA) is a metabolic reprogramming agent that targets the mitochondrial pyruvate dehydrogenase complex (PDC) which sits at the nexus of carbohydrate, lipid and amino acid metabolism. By activating the PDC, treatment with DCA effectively diverts tumor cells away from Warburg-induced aerobic glycolysis back toward a more oxidative metabolism. This "normalization" of tumor metabolism proves lethal to the tumor cell. Here we show that this same metabolic reprogramming has beneficial effects in mitigating tumor-induced muscle wasting. *In vitro* studies were conducted wherein C2C12 myotubes were treated with different percentages of conditioned media (CM, 10%, 25% and 50%) from C26 and ES-2 cachexia-inducing tumor cells with or without the addition of 1 mM DCA. The data show that the CM in each case, induces myotubes atrophy in a dose dependent manner. DCA treatment partially rescue myotubes size in all the groups. (+9%, +14%, +10% *vs.* C26 CM 10% 25% and 50% respectively; $p<0.05$ and (+14%, +20%, +10% *vs.* ES-2 CM 10%, 25% and 50% respectively; $p<0.05$). Preliminary results from an *in vivo* model of DCA treatment of cancer cachexia have also demonstrate a reduction in skeletal muscle wasting. In this study, mice were implanted with C26 adenocarcinoma cells. Muscle wasting was observed around day eight followed by a precipitous decline in weight until sacrifice at day 12. The treatment group were given DCA (500 mg/kg) in the drinking water starting at day four and continuing until sacrifice at day 12. The final weights of the gastrocnemius and tibialis muscles were significantly higher in the treated group compared with controls. These results demonstrate the potential of a metabolic reprogramming to protect muscle from cancer-induced cachexia. Future studies will examine dose-response effects of DCA in both muscle and tumor and include different models of cancer-induced cachexia.

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

POSTER #46

MODULATION OF PDK4 DRIVES METABOLIC ALTERATIONS AND SKELETAL MUSCLE ATROPHY DURING CANCER CACHEXIA

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Cancer contributes to a persistent imbalance in the use of energy substrates, often leading to severe metabolic dysfunctions and to the occurrence of a devastating complication known as 'cachexia'. We and others have shown that mitochondrial dysfunctions and shift from oxidative-to-glycolytic metabolism may contribute to skeletal muscle atrophy due to cancer.Despite recent progress in the identification of therapies for cancer, no treatments have been approved thus far for cachexia, and the mechanisms responsible for this debilitating condition remain poorly described.

An important regulator of cellular energetic metabolism is the pyruvate dehydrogenase kinase-4 (PDK4), an inhibitor of the pyruvate dehydrogenase (PDH) complex, sitting at the nexus of three critical metabolic pathways, glycolysis, TCA cycle and ß-oxidation. Previous studies revealed that PDK4 is elevated in skeletal muscle in conditions of starvation and diabetes, while its pharmacologic inhibition leads to a protective effect against statin-mediated myopathy. Regardless, whether PDK4 is directly involved in the regulation of skeletal muscle size and function remains to be elucidated. Hence, the aim of our study was to investigate the metabolic link between PDK4 activation and changes in muscle size and function in the context of cancer-associated cachexia.

Here we show that growth of the murine C26 colorectal adenocarcinoma promotes severe skeletal muscle wasting and metabolic derangements consistent with higher PDK4 expression (+5-fold *vs.* Control, $p<0.01$), as well as reduced PDH (-23% *vs.* Control; $p<0.05$) and succinate dehydrogenase (SDH; -53% *vs.* Control; $p<0.01$) enzymatic activities. Similarly, *in vitro* studies using C2C12 myotubes exposed to 25% C26 conditioned medium for up to 48 hours showed reduced myofiber size (-29% vs. Control; $p<0.001$), as well as increased PDK4 levels (+28% vs. Control; $p<0.05$). Conversely, adenoviral-induced PDK4 overexpression in C2C12 cultures was sufficient to promote significant myofiber atrophy (-24% *vs.* Control; $p<0.001$), accompanied by a dramatic decrease in oxygen consumption rate (OCR). Analogously, mice fed a diet enriched in WY-14643, a PDK4 activator, displayed reduction in fat and lean mass, also consistent with muscle atrophy and decreased muscle SDH activity (-20% *vs.* Control; $p<0.05$). Interestingly, dichloroacetate (DCA; 1 mM), a well-known PDK4 inhibitor, reduced the phospho-PDH/PDH ratio (-55 % *vs.* C26; $p<0.05$) and partially rescued myofiber size (+20% *vs.* Control; $p<0.05$) in C2C12 cells exposed to C26 conditioned medium. DCA administration also improved muscle mass in C26-bearing mice, despite no change in tumor size (+33% vs. C26; $p<0.01$).

Overall, our data support the idea that PDK4-mediated metabolic alterations in the skeletal muscle play a direct role in promoting cancer-associated muscle atrophy. Further studies will aim at evaluating whether PDK4 pharmacological inhibitors, alone or in combination with chemotherapy, may contribute to the improvement of muscle phenotype and overall quality of life in patients with cancer.

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

POSTER #47

DUAL TGFB/BMP INHIBITION ALLOWS IN VITRO EXPANSION OF MULTIPLE CELL TYPES FROM NORMAL AND CANCEROUS BREAST

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Functional modeling of breast epithelial hierarchy and stromal-epithelial cell interactions has been difficult due to inability to obtain sufficient stem-progenitor-mature epithelial cells and stromal cells. The recently developed epithelial reprogramming assay has partially overcome this limitation, allowing propagation of epithelial cells with stem, luminal progenitor and mature cell features. However, characterizing stromal cells using this assay is difficult because irradiated fibroblasts which can be difficult to distinguish from stromal cells are needed as feeder layer. A recent study demonstrated expansion of airway basal stem cells without a feeder layer through pharmacologic inhibition of TGFb/BMP/SMAD signaling. We sought to develop this method for culture and expansion of cells from normal and cancerous breast samples. With appropriate modifications to growth media, we were able to obtain normal and stromal cells from breast biopsies of healthy women. The expanded cell population included CD10+/EpCAM- basal/myoepithelial cells, CD49f+/EpCAM+ luminal progenitor cells, CD49f-/EpCAM+ mature luminal cells, CD73+/EpCAM+/CD90- rare endogenous pluripotent somatic stem cells, CD73+/CD90+/EpCAM- mesenchymal stem cells, ALCAM (CD166)+/EpCAM+ cells, CD44+/CD24- cells, CD44+/CD24+ cells and ALDFLUOR+ stem/luminal progenitor cells. Epithelial cells were KRT14+, KRT19+ or both further documenting heterogeneity within epithelial cell population. We have extended this technique to grow breast epithelial cells from high-risk patients including BRCA1 mutant-carriers, tumor-adjacent normal and tumor cells from the same patient, pleural effusions and liver metastasis from breast cancer patients. Phenotypic characterization showed differences in the differentiation state of adjacent-normal and tumor cells. Tumor cells from pleural effusions showed remarkable phenotypic heterogeneity with a fraction of these cells expressing estrogen receptor. The assay described here, therefore, is versatile and provides resources to model epithelial-stromal interactions under normal and cancerous conditions as well as for genomics and screening of drugs to target metastasis on an individual level.

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

POSTER #48

DEVELOPING HSP60/10 CHAPERONIN INHIBITORS FOR COLORECTAL CANCER CHEMOTHERAPY

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Colorectal cancer (CRC) is the third most prevalent cancer in both males and females, accounting for ~10% of all cancers and adding nearly one million new cases worldwide annually. Despite the improvement of early stage detection and treatment, CRC is still the third leading cause of cancer-related death, highlighting the need of novel target and treatment. During the past few years, heat shock protein family emerges as potential target in different cancers. This family is involved in protein folding and maturation, insuring proper cell functions. The 60 kDa Heat Shock Protein (HSP60) is the major chaperone system in eukaryotic mitochondria. It has recently been described as a potent target in different cancer including colorectal cancer. Studies show that HSP60 functions are associated with apoptotic, pro-survival, and metastatic pathways (e.g. modulation of p53, Bax, cytochrome c, survivin, IKK/NF-¿B, ß-catenin, a3ß1 integrin, and HIF-1a). While the role of HSP60 in oncogenesis is not fully understood, cytosolic accumulation of HSP60 in tumor environments with or without mitochondrial release is likely to contribute towards tumor progression. Here we propose to develop HSP60 inhibitor able to target selectively cytosolic form of HSP60. We hypothesis that targeting cytosolic HSP60 and not mitochondrial one will decrease the potent cytotoxicity of these compounds on non cancerous cells. We previously realized a high-throughput screening of ~700,000 compounds, leading to the identification of several hundred potent chaperonin inhibitors, which block the refolding function of HSP60. Many of these initial hits and first generation analogs inhibit selectively the growth of colon cancer cell lines (HCT-116, HT-29 and DLD-1) compared to non cancerous cells. We are currently optimizing these HSP60 inhibitors for *in vivo* chemotherapeutic efficacy testing, and use these compounds as a new tool to elucidate HSP60 implication in colorectal carcinogenesis.

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

POSTER #49

OXIDATIVE DAMAGE-INDUCED EPIGENETIC CHANGES IN INTESTINAL STEM CELLS AND TUMORIGENESIS.

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Colorectal cancer (CRC) is the second leading cause of cancer related deaths. Inflammation is an important risk factor associated CRC. To study inflammation-induced tumorigenesis we infect multiple intestinal neoplasia (Min) mice with enterotoxigenic *Bacteroides fragilis* (ETBF), which results in acute inflammation, leading to chronic colitis and tumorigenesis. ETBF has been associated with diarrheal disease, inflammatory bowel disease and CRC in humans. Previously our lab has shown that two days post ETBF infection high levels of oxidative damage induce an epigenetic response that results in promoter enrichment of trimethylated histone H3 lysine 27 (H3K27me3) catalyzed by enhancer of zeste homolog 2 (EZH2) and reduced expression of a subset of tumor suppressor genes (TSGs) in the intestinal epithelium when compared to epithelium from uninfected (mock) mice. We further demonstrated that this acute epigenetic response was necessary for DNA hypermethylation and reduced expression of these TSGs in tumors that are induced by the inflammation. Interestingly, the expression of the TSGs had returned to normal levels in the bulk epithelium seven days after infection. Therefore, it is yet not understood, which cells in the colon retain the initial inflammation-induced epigenetic repression seen at the 2 day time point and what are the steps resulting in DNA methylation at the promoters of these TSGs during tumorigenesis. The colon epithelium is regenerated every 5-7 days from Leucine-rich-repeat-containing G-protein coupled receptor 5 (Lgr5+) stem cells, suggesting that stem cells are the cells that are exposed to the initial oxidative damage and persist through tumorigenesis. Thus, we hypothesize that acute inflammation induces epigenetic silencing of TSGs in exposed adult stem cells, which persist and result in DNA methylation and tumor formation. Lgr5+ cells sorted from epithelium of mice 2 days post-ETBF have decreased expression of the TSGs compared to similar cells from mock-infected mice. Caudal-related homeobox transcription factor, *Cdx1*, is a TSG that has promoter CpG island DNA hypermethylation and decreased expression in ETBF tumors and in ETBF-exposed LGR5+ cells. CDX1 is an important regulator in CRC stem cells and inhibits the polycomb complex ring finger protein, *Bmi1* via mir215. BMI1 is overexpressed in CRC patients and correlates with poor patient survival. *Bmi1* is upregulated in ETBF tumors by RNA sequencing. *Bmi1* levels are increased in ETBF-stem cells. Lgr5+ stem cells form organiods in 3-D tissue culture and serve as a practical model to study stem cells *in vitro*. Organoids derived from ETBF mice appear larger and exhibit increased proliferation and differentiation, which may be due to decreased *Cdx1* and increased *Bmi1* levels. Unraveling the mechanism by which epigenetic changes persist in stem cells and affect downstream signaling could help in development of intervention strategies to reverse inflammation-induced epigenetic changes and reduce tumorigenesis and overall improve human health.

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

POSTER #50

ERROR-PRONE MITOSIS DUE TO IMPAIRMENT OF THE NOVEL FANCA-BRCA2-BUBR1 SPINDLE CHECKPOINT SIGNALING AXIS

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Fanconi anemia (FA) is an inherited bone marrow failure syndrome associated with genomic instability, malignancies such as acute myeloid leukemia (AML) and congenital skeletal malformations. Somatic mutations within the FA/BRCA signaling network occur in AML in the general population, reflecting the importance of FA genes in tumor suppression. While the role of FA proteins in interphase DNA damage repair (DDR) and replication is well-established, we and others found that the FA network is essential for error-free chromosome segregation during cell division. However, the molecular mechanisms of FA pathway-dependent genome housekeeping during mitosis are incompletely understood. Further, it is unclear whether error-prone mitosis contributes to the development and progression of FA-associated leukemias in vivo.

To understand how loss of FANCA affects cell signaling and identify candidate therapeutic targets, we first employed a functional genomics approach to identify signaling networks essential for FANCA-/- patient cell survival. Through a synthetic lethal kinome-wide shRNA screen in FANCA patient cells, we discovered interphase and mitotic phosphosignaling networks that FANCA-/- cells depend on for survival, including the BUB1-BUBR1 axis of the spindle assembly checkpoint (SAC). BUB1 and BUBR1 are essential SAC kinases that prevent chromosome mis-segregation by inhibiting the APC (anaphase-promoting complex) ubiquitin ligase at the centromeres until all kinetochores are correctly attached to the spindle microtubules. Our super-resolution microscopy and biochemistry experiments revealed that FANCA shuttles to kinetochores upon mitotic entry and physically interacts with BUB1 and BUBR1 at the kinetochore-microtubule attachment sites in attachment- and tension-dependent manner. We found that FANCA is essential for BUBR1 lysine-250 (K-250) acetylation at prometaphase kinetochores, and we confirmed that endogenous BUBR1K250 acetylation is disrupted in FANCA-/- primary patient cells. BUBR1K250 acetylation event works as a molecular switch in which BUBR1 is converted from a degradation target to a potent inhibitor of the APC ligase. Further, loss of FANCA disrupted kinetochore recruitment of the BUBR1K250 acetyltransferase PCAF and its upstream regulator, FANCD1/BRCA2.

Together, our findings establish the first mitotic mechanistic connection between FANCA, the canonical SAC tumor suppressor cascade, and the FA effector FANCD1/BRCA2. These findings further our understanding of the mechanisms of genomic instability resulting from loss of FA signaling and suggest candidate therapeutic targets based on our synthetic lethality screen results.

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

POSTER #51

PROTEOMIC SCREENING REVEALS THE PARYLATION LANDSCAPE OF BASE EXCISION REPAIR PROTEINS AFTER ¿-LAPACHONE TREATMENT IN COLORECTAL CANCER CELLS

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Colorectal cancer (CRC) is a leading cause of cancer death and the third most common type of cancer worldwide. In early cases, surgery is the mainstay of treatment, but often patients are primarily diagnosed in an advanced stage of disease and may have distant metastases. Most solid tumors overexpress NAD(P)H;Quinone Oxidoreductase 1 (NQO1), which catalyzes a futile redox cycle of b-lapachone during exposure, resulting in the formation of high levels of reactive oxygen species (ROS). Futile cycle b-lapachone recycling produces elevated superoxide levels, resulting in massive levels of hydrogen peroxide that lead to substantial DNA damage and endoplasmic reticulum Ca^{2+} release. DNA damage and Ca+2 entry into the nucleus leads to the hyperactivation of poly(ADP-ribose) polymerase 1 (PARP1). A majority of parylation is noted when hyperactivated PARP1 self-parylates, forming extensive Poly(ADP) ribosylated PAR-PARP1 levels are noted within 5 mins of exposure to 4 µM ß-lap in A549 cells, as noted by Western blotting using an antibody specific for PARylated moieties. PAR-PARP1 is a protein post-translational modification catalyzed by a family of enzymes known as PARPs. DNA single strand breaks along with base-damage and abasic sites together with nuclear Ca^{2+} levels lead to hyperactivation of PARP1, a DNA repair enzyme that utilizes NAD+ to generate PAR moieties. This is followed by dramatic NAD+/ATP losses and tumor cells die of a special programmed necrosis process, referred to as NAD+-keresis.

PAR-PARP1 is rapidly degraded by poly(ADP-ribose) glycohydrolase (PARG) a exo- and endoglycosidase. **PARG-knockdown HCT116 cells were used to gain insight into the function of PARG and whether PARG depletion would enhance b-lapachone induced cell death by blocking PAR recycling.** Proteomic screening after b-lapachone treatment in colorectal cancer cell line revealed the PARylation landscape of base excision, mismatch and nucleotide excision repair proteins. Twenty (20) of the proteins were found to overlap with H_2O_2 treatments (200 µM or 2 mM), while one protein (i.e., SPSF) was unique to b-lapachone treatment. b-Lapachone treatment affects BER pathways and qualitative analysis of the human Asp- and Glu-ADP-ribosylated proteome after b-lapachone treatment indicate major alterations in BER pathways. We confirmed that b-lapachone mediated cytotoxicity was independent of PARG knockdown or TOPO I or II protein losses. In contrast, RFC1 and PCNA siRNA-mediated protein knock downs significantly enhanced b-lapachone lethality, whereas knockdown of Topo I or Topo IIalpha did not affect b-lapachone lethality. Taken together, these results demonstrated that knockdown of RFC1 enhances lethality of b-lapachone and could serve as an effective treatment strategy in CRC. We are currently delineating the mechanism of enhanced lethality of ß-lapachone by loss of RFC1. *This work was funded by NIH/NCI* ***5 R01 CA102792-16*** *to DAB.*

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

POSTER #52

GLUCOSE METABOLISM AND INSULIN RELEASE ARE IMPAIRED UNDER CONDITIONS OF EXCESS TGF-ß MEDIATED HIGH BONE TURNOVER

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Bone loss and/or type 2 diabetes increases the risk of pathological fractures, muscle weakness and obesity. We have shown that TGF-ß released from the bone matrix during resorption can act systemically to cause skeletal muscle weakness. Given that circulating TGF-ß and other bone-derived factors can potentially signal in different tissues, we explored the significance of bone-to-pancreas signaling using a mouse model of Camurati-Engelmann disease (CED), a rare bone diaphysial dysplasia associated with high bone turnover. Mice expressing a bone-directed CED mutation exhibit high bone resorption, increased circulating TGF-ß, fractures, and muscle weakness. Since TGF-ß can impair insulin secretion, we hypothesized that the high bone turnover and increased systemic TGF-ß in CED mice would cause hyperglycemia due to impaired insulin secretion from pancreatic ß-cells. Forty-five-week old control and CED mice were fed either high fat diet (HFD) or low fat diet (LFD) for 15 weeks, mimicking the conditions of aging, high bone resorption, and obesity. Our data show that HFD CED mice have higher fat mass compared to HFD control ($p<0.001$) suggesting that high bone resorption and release of bone-derived factors exacerbate the effects of diet-induced obesity. Compared to HFD controls, HFD CED mice had significant glucose intolerance ($p<0.01$) and mild insulin resistance. Isolated pancreatic ß-cells from HFD CED mice showed significantly less insulin secretion ($p<0.05$) in response to glucose stimulation, indicating impaired metabolic-secretion coupling. In contrast, there were no differences in glucose tolerance, insulin sensitivity, insulin secretion, or fat mass between control and CED mice on LFD. Muscle specific force in HFD CED mice was significantly lower compared to HFD control ($p<0.001$). The muscle specific force for CED LFD was lower compare to control but was not as reduced as that seen with the HFD. This implies that muscle weakness in the setting of CED-induced high bone turnover is sensitive to the added insult of diet-induced obesity and its associated metabolic complications. Overall, our data indicate that a state of high bone turnover (with excess TGF-ß) adversely affects both glucose and fat metabolism and impairs insulin secretion by pancreatic ß-cells. Future studies will be directed at understanding the mechanisms of bone-energy crosstalk to develop therapeutic targets in conditions of bone loss associated with diabetes, obesity, and age related sarcopenia.

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

POSTER #53

SOMATIC MUTATION OF THE COHESIN COMPLEX SUBUNIT CONFERS THERAPEUTIC VULNERABILITIES IN HUMAN CANCER

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Synthetic lethality-based strategy enables the identification of potential therapeutic targets in cancers harboring tumor suppressor gene mutations. Previous success was achieved using poly-ADP ribose polymerase (PARP) inhibitors in BRCA1/2-mutated tumors. A major deadlock in this approach is due to the fact that such genes usually do not perform fundamental or indispensable functions in the cell. We identified the "essential lethality" that arose from mutated/deleted essential genes, which are largely tolerated in cancer cells due to genetic redundancy between paralog genes. Using this approach we uncovered the cohesion subunit stromal antigen 1 (STAG1) as a putative synthetic-essential target in cancers carrying inactivating mutations of its paralog, STAG2. In STAG2-deficient Ewing sarcoma and bladder cancer, further depletion of STAG1 suppressed cancer cell proliferation, survival and tumorigenic potential. Mechanistically, inhibition of STAG1 in STAG2-mutated cells leads to premature chromatid separation, dramatic extension of mitotic duration, and consequently lethal failure of cell division. More importantly, depletion of STAG1 renders those STAG2-mutated cells more susceptible to DNA damage, especially double-strand breaks, due to reduced functionality of DNA repair. Thus, inhibition of STAG1 sensitizes STAG2-deficient cancer cells to PARP inhibitors. These *in vitro* findings were further validated using orthotopic tumor models of bladder cancer and sarcoma. In conclusion, our study highlights a potential therapeutic strategy for patients with STAG2-deficient tumors.

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

POSTER #54

FUNCTIONAL VALIDATION OF A GENOME-WIDE ASSOCIATION STUDY (GWAS) SNP ASSOCIATED WITH ANTHRACYCLINE-INDUCED CONGESTIVE HEART FAILURE

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Anthracyclines are commonly used chemotherapies for the treatment of breast cancer, but they can cause dose-related cardiotoxicities and lead to congestive heart failure (CHF) in ~2% of patients. There are no clinically available predictive biomarkers for this toxicity. Previously we have identified and validated the association of a single nucleotide polymorphism (SNP), *rs28714259*, with CHF in three large adjuvant phase III breast cancer trials. *rs28714259* locates in a putative glucocorticoid receptor (GR) binding site and the risk (minor) allele is predicted to disrupt GR binding. Interestingly, activation of GR-signaling by dexamethasone has been shown to protect cardiomyocytes from doxorubicin-induced apoptosis in rat cardiomyocytes. To test whether *rs28714259* presents allele-specific enhancer activity on GR-mediated transcriptional regulation, we cloned a ~2kb sequence surrounding *rs28714259*, with either the major (G) or minor (A) allele, to a luciferase reporter plasmid. MCF-7 cells transfected with major allele-luciferase construct demonstrated a 1.5-fold increase in luciferase activity 1 hour after 100nM dexamethasone treatment compared to vehicle control, whereas no increase was observed in cells transfected with the minor-allele construct. We next tested whether the impairment in enhancer activity associated with the minor-allele was due to decreased binding affinity to GR by electrophoretic mobility shift assay (EMSA). 100nM of dexamethasone for 1 hour in MCF-7 cells induced a prominent band shift in either the major- or risk-allele probes. Notably, we observed a 60% reduction in the shifted band intensity of risk-allele probes compared to major-allele probes, suggesting decreased GR binding affinity to the risk-allele. Addition of GR antibody to the binding reaction resulted in a detectable “supershift” which confirmed the protein binding to *rs28714259* probes is indeed GR. Consistently, the supershift band intensity of minor allele-probes was decreased by over 60% compared to major allele-probes. Taken together, our results suggest that minor allele of *rs28714259*increases risk of anthracycline-induced CHF through disrupting GR-mediated transcriptional regulation. Further validations are ongoing using cardiomyocytes derived from human induced pluripotent stem cells (iPSCs). These studies provide further insight into the function and role of *rs28714259* in anthracycline-induced CHF.

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

POSTER #55

THE ROLE OF PTEN AND DICER HAPLOINSUFFICIENCY IN ENDOMETRIAL CANCER DEVELOPMENT

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Endometrial cancer is the most frequent gynecologic malignancy, with 61,380 new cases diagnosed in 2017 in the US. While women with early stage disease are typically cured with simple hysterectomy, the prognosis for women with aggressive forms of the disease (*i.e.,* clear-cell adenocarcinoma or carcinosarcoma) is dismal. To study endometrial cancer in a mouse model, we started with the $Pgr^{Cre};Pten^{f/f}$ mouse (*Pten* cKO). This mouse model deletes *Pten*, the most frequently mutated tumor suppressor in women with endometrial cancer, using a conditional allele for the uterus. To this mouse model, we further deleted one or two alleles of *Dicer* [$Pgr^{Cre};Pten^{f/f};Dicer^{f/+}$ *(Pten-Dicer* het*)* and $Pgr^{Cre};Pten^{f/f};Dicer^{f/f}$ (dcKO)]. DICER is a ribonuclease III required for miRNA synthesis, and lowed DICER expression correlates with poor prognosis tumors and recurrent disease in women. Surprisingly, *Pten-Dicer* het female mice (n=19) had significantly worse survival than *Pten* cKO (n=22) or dcKO (n=19) [P<0.05]. Detailed histological examination of uterine tumors from all genotypes of survival mice showed adenocarcinomas with high-grade features. Interestingly, we found two dcKO mice with malignant mixed *Müllerian* tumor (MMMT). MMMT of the uterus, also known a uterine carcinosarcoma, is an extremely rare and aggressive malignancy in women. To determine the mechanism of the more aggressive disease in *Pten-Dicer* het female mice, we performed RNA and small RNA sequencing. Comparison of RNA-seq analysis from early age mice to data from women via The Tumor Cancer Genome Atlas (TCGA) revealed clustering of all mouse models into the *POLE* ultramutant tumor types. Interestingly, *Pten* cKO and *Pten-Dicer* het transcriptomic profiles were enriched in immunoreactive signatures, while dcKO profiles were enriched in hormonal signatures. MiRNA-seq analysis revealed 52 miRNA molecules dysregulated in *Pten-Dicer* het uteri and 100 miRNA molecules dysregulated in dcKO uteri compared to *Pten* cKO (P<0.001, fold-change 1.2). To complete characterization, we examined steroid hormone dependence of the tumors. Uterine weights of ovarectomized mice were smaller across all genotypes compared to intact mice at 12 weeks (P<0.001), suggesting hormone-dependent tumors. Survival studies with ovariectomized mice showed overall increased survival in all genotypes compared to intact female mice. However, the histology of hormone-depleted tumors was surprisingly more aggressive from the intact mice. Both *Pten* cKO and *Pten-Dicer* het female mice had an increased frequency of clear-cell adenocarcinomas in uterine tumors after ovariectomy. Mouse models of endometrial cancer that recapitulate human disease represent translational tools for better understanding of aggressive disease. We anticipate that this model will be highly relevant, not only to study the molecular characteristics of the more rare forms of human endometrial cancer, but also to study the preclinical development of therapeutics for uterus carcinomas.

Basic Science ***Research Associate***

POSTER #56

CHRONIC TREATMENT WITH MULTI-KINASE INHIBITORS PROMOTES GROWTH RETARDATION AND CACHEXIA-LIKE SYMPTOMS

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Despite the recent progress in the development of new therapies for cancer, chemotherapy remains the preferred treatment strategy for most tumors, irrespective of its associated toxicities. We recently showed that commonly used anticancer drugs contribute to the development and sustainment of cachexia, a complication of cancer, mainly characterized by loss of body weight and depletion of muscle and fat tissue. No treatments have been approved thus far for the management of cachexia, and the mechanisms responsible for this debilitating condition remain only partially described. Multi-kinase inhibitors, such as regorafenib and sorafenib, recently emerged as second-line molecular targeted strategies to improve the efficacy of classical chemotherapy in the treatment of advanced solid tumors. However, common side toxicities, including body weight loss, muscle weakness and fatigue, often represent challenging complications affecting quality of life and overall outcomes in patients with cancer.

The goal of the present study was to characterize the causes responsible for abnormal skeletal and cardiac muscle size and function in animals exposed to regorafenib and sorafenib for up to 6 weeks. CD2F1 male mice (8-week old; n=5-6) were treated intraperitoneally (i.p., daily) with regorafenib (30 mg/kg) or sorafenib (60 mg/kg). Reduced body and carcass weights, as well as decreased skeletal and cardiac muscle size were evident in the animals receiving either of the two compounds when compared to the vehicle-treated mice. Consistently, administration of the two inhibitors was accompanied by muscle weakness, as suggested by the measurement of grip strength and ex-vivo muscle contractility. Modulation of ERK1/2, P38 and GSK3¿, signaling pathways often associated with muscle atrophy conditions, was reported in the skeletal muscle of the treated animals. On the other hand, enhanced activation of autophagy markers, such as LC3B-II and beclin 1, was shown upon treatment with regorafenib or sorafenib, while no change in the expression of mitochondrial regulators, such as PGC1¿ and OPA1, was reported. Notably, exposure to these multi-kinase inhibitors also promoted physiological changes in the heart by significantly reducing left ventricular mass, internal diameter, posterior wall thickness and stroke volume, despite unchanged overall function.

In conclusion, our results suggest that chronic administration of the multi-kinase inhibitors regorafenib and sorafenib determines growth retardation associated with cachexia-like symptoms, including abnormalities in skeletal and cardiac muscles. Altogether, these events may concur to the occurrence of muscle weakness and cardiac deficits. Effective pharmacologic interventions making use of multi-kinase inhibitors will therefore need to take into account these complications in order to determine fewer chemotherapy toxicity and better outcomes in patients affected with cancer.

Basic Science ***Research Technician***

POSTER #57

TRANSCRIPTIONAL MODIFICATIONS IN BREAST CANCER INITIATION

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Background: In the search for new preventive strategies, determining the molecular mechanism of breast cancer initiation is critical. While previous reports have shown expressional changes in either pre-malignant (i.e. DCIS) or advanced lesions, we describe here the transcriptomic analysis of specimens (1-4 years) before any clinical diagnosis of the disease (cancer precursor). In the present work, we report on the transcriptome differences in the microdissected breast epithelium of cancer precursors versus healthy premenopausal women.

Methods: The specimens were obtained from the Susan G. Komen Tissue Bank at IU Simon Cancer Center. We compared the transcriptome profiles of breast tissues from 7 susceptible and 16 healthy premenopausal women between the age of 34 and 52 years, who were free of breast cancer pathology at the time they donated the tissue. Donors in the two experimental groups were matched according to age, racial background and menstrual phase. Differential expression analysis was performed using edgeR_Bioconductor. False discovery rate (FDR) was computed from p-values using the Benjamini-Hochberg procedure. Ingenuity Pathway Analysis was used to identify relevant signaling pathways. Transcriptome differences were examined independently from the menstrual phase to eliminate breast epithelium gene expression variations affected by circulating hormones during menstrual cycle.

Results/Discussion: We found 362 transcripts differentially expressed between the two groups ($p<0.05$). However, only 285 changed independently from the follicular or luteal status. Among the upregulated genes, we observed three major affected pathways: 1) lipid metabolism, 2) molecular transport, and 3) energy production. A limited number of genes were highly differentially expressed between susceptible samples and healthy controls ($p<0.05$, fold change> 2 and FDR<0.2); among those we identified genes involved in cellular metabolism (AKR1C1), cytoskeleton organization (XIRP2) and glycerol transport (AQP7). Interestingly, the transcription repressor ZFP57 was found highly downregulated (fold change=-29; $p=0.0002$; FDR=0.09).

The upregulation of AK1RC1 in the breast epithelium of cancer precursors as compared with healthy controls, was confirmed by qPCR (fold change=4.3, $p=0.02$). AKR1C1, aldo-keto reductase C1, is an enzyme involved in maintaining steroid hormone homeostasis, prostaglandin metabolism, and metabolic activation of polycyclic aromatic hydrocarbons. Moreover, previous report show that AKR1C1's expression is altered in breast cancer and it plays a role in cancer invasion and chemo resistance.

Conclusion: This study shows that early stages of breast cancer display transcriptional alterations in metabolic pathways as well as changes in transcriptional regulations. These findings will contribute to a better understanding of the mechanisms of cancer initiation, as well as the identification of new therapeutic targets.

Basic Science ***Research Technician***

POSTER #58

DEVELOPMENT OF A HIGH-THROUGHPUT BIOASSAY TO FUNCTIONALLY TEST INTRONIC GENETIC VARIANTS PREDICTED TO ALTER PRE-MRNA SPLICING

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Background: Clofarabine, a chemotherapeutic agent used to treat relapsed or refractory lymphocytic leukemia shows significant inter-individual variability in drug induced cytotoxicity. Many intronic single nucleotide variants (iSNVs) were found to be associated with clofarabine induced cytotoxicity. These iSNVs can change gene function by altering pre-mRNA splicing. We utilized a machine-learning based bioinformatic approach to prioritize iSNVs associated with clofarbine toxicity based on their potential to change splicing and protein function. A need exists for a high-throughput bioassay to functionally test these large number of iSNVs. Therefore, we developed a high-throughput bioassay that can functionally test several iSNVs in parallel.

Methods: 706 iSNVs associated with clofarabine-induced cytotoxicity were informatically prioritized based on their potential to alter pre-mRNA splicing and influence protein function. Oligonucleotides with a 20 nucleotide exon fragment and a 50 nucleotide intron fragment containing the wild-type or variant allele of the iSNV were synthesized as a pool of 40 prioritized test iSNVs, and 16 positive control sequences. These oligonucleotides were inserted into an ExonTrap plasmid in parallel to generate a plasmid library, which was transfected into HeLa, HEK293, HepG2, and LCL cells; total RNA was isolated and cDNA prepared. The transcripts from each cell line were amplified using barcoded universal PCR primers. Barcoded PCR products were pooled and sequenced using Illumina's NextSeq 500 platform. Spliced Transcripts Alignment to a Reference (STAR) algorithm was used to quantify splice isoforms. The impact of iSNVs on splicing outcome was determined by comparing the ratio of read counts supporting perfectly spliced to aberrantly spliced transcripts for each allele. Significant iSNVs were determined by Mann-Whitney-Wilcoxon test and FDR corrected p-values <0.05.

Results: Using our high-throughput bioassay, 27 out of 40 test iSNVs were found to be functional. In 18 of the 27 functional iSNVs the variant allele disrupted the splice donor site, producing a smaller proportion of perfect splice products compared to the wild-type allele. E.g., in rs7079830 there is a 44.6 – 54.3% decrease in perfectly spliced transcripts in all cell lines. In 9 functional iSNVs, the variant allele created a new splice donor site, producing a greater proportion of perfect splice products compared to the wild-type allele. E.g., in rs62375235 there is a 15.2-105.1% increase of perfectly spliced transcripts in all cell lines. We observe a highly consistent impact of individual iSNVs across multiple cell lines. Out of 16 positive control iSNVs with previous experimental evidence of altered splicing, 14 were identified as functional using our assay.

Conclusion: Using our novel high-throughput assay we have identified 27 functional iSNVs associated with clofarabine-induced cytotoxicity. We will further validate these iSNVs using individual *in vitro* splicing assays and by *in vivo* cytotoxicity assays using genotyped International HapMap LCL cells.

Basic Science ***Research Technician***

POSTER #59

REDUCTION OF TUMOR BURDEN AND HEARING LOSS WITH A MULTIPLE RECEPTOR TYROSINE KINASE INHIBITOR BRIGATINIB IN A GENETICALLY ENGINEERED MOUSE MODEL OF NEUROFIBROMATOSIS TYPE 2

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Neurofibromatosis Type 2 (NF2) is an autosomal dominant genetic disorder caused by germline mutations in the tumor suppressor gene *NF2*, which encodes the protein Merlin. Patients with NF2 develop bilateral vestibular schwannomas, which cause hearing impairment, and may also develop schwannomas in other regions of the peripheral nervous system, as well as astrocytomas, ependymomas, and meningiomas. Due to the nature and location of the tumors, there is high morbidity and mortality associated with NF2, and no known effective chemotherapeutic intervention. We utilized a high-throughput *in vitro* cell viability screen to evaluate the effects of 19 different drugs in both *Nf2*-deficient and *Nf2*-sufficient Schwann cell cultures. This screen revealed an Anaplastic Lymphoma Kinase (ALK) inhibitor as a top contender for use as a single agent. The ALK inhibitor also exhibited synergistic activity with several other drugs used in the screen and appeared to have selectively inhibited the *Nf2*-deficient Schwann cells. Therefore, we hypothesized that ALK inhibition would prevent tumorigenesis in our NF2 GEMM ($Nf2^{f/f}$; *Postn-Cre*+).

Brigatinib (Alunbrig®) is a small molecule multiple receptor tyrosine kinase inhibitor that is FDA-approved for ALK-positive metastatic non-small cell lung cancer. Brigatinib was administered to $Nf2^{f/f}$; *Postn-Cre*+ mice by oral gavage at a dose of 50mg/kg once per day for 12 weeks, beginning at four months of age. Hearing loss was assessed by Auditory Brainstem Response (ABR) at the beginning and end of treatment. At the end of the therapeutic trial, blood and relevant tissues were collected, and tumorigenic tissues were processed for histological analysis. The volumes of dorsal root ganglia (DRG), sites of schwannomagenesis in $Nf2^{f/f}$; *Postn*-Cre+ mice, were calculated using the formula for the approximate volume of a spheroid (volume = length x $(\text{width})^2$ x 0.52).

Pharmacokinetic analyses paired with immunohistochemistry showed that we were able to achieve a therapeutically relevant concentration of brigatinib in the plasma (c_{max} = 4.8μM; t_{max} = 4hrs), and we were able to induce a biochemical response in the tumors. The volume of DRG in brigatinib-treated mice was decreased 55% compared to vehicle-treated mice. Additionally, there was no significant difference between ABR thresholds of brigatinib-treated mice at the end of treatment when compared to baseline, whereas vehicle-treated mice had a statistically significant 7.4dB increase in ABR threshold. Finally, histopathological assessment of DRG shows that brigatinib treatment prevented the progressive tissue disruption observed in the vehicle-treated mice, and that the tissue architecture of brigatinib-treated mice was comparable to four month

old untreated *Nf2*$^{f/f}$; *Postn-Cre+* mice. These data provide evidence that brigatinib targets may play a significant role in Merlin-deficient schwannomagenesis, and implicate brigatinib as a promising therapeutic intervention of NF2.

Basic Science ***Research Technician***

POSTER #60

TARGETING THE DNA DAMAGE RESPONSE FOR CANCER THERAPY VIA SMALL MOLECULE INHIBITION OF REPLICATION PROTEIN A-DNA BINDING ACTIVITY

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The DNA damage response (DDR) is a tractable pathway to target for the treatment of cancer. The inherent replication stress (RS) experienced by rapidly dividing cancer cells provides a therapeutic window for anticancer activity of agents targeting the DDR. In addition, inhibiting the DDR can enhance the activity of common chemo-and radio-therapeutic interventions that impart their clinical efficacy via the induction of DNA damage. The DDR is initiated by engagement of the of PI3 kinase-related kinases ATM, ATR, and DNA-PK. These proteins and the downstream checkpoint kinases, CHK1 and CHK2, are validated targets pursued clinically. Oncogenic RS coupled with DDR blockade results in local effects at the replication fork and global effects on cell cycle and signaling which ultimately result in replication catastrophe (RC) and cell death. The human single stranded DNA (ssDNA) binding protein, replication protein A (RPA), is a critical regulator of RC with depletion of RPA or "RPA exhaustion" driving RC and cell death. High levels of ssDNA exhaust cellular RPA such that there is insufficient RPA-DNA binding capacity to engage all the ssDNA generated. The lack of RPA then renders DNA susceptible to nuclease digestion, RC and cell death. We have discovered and developed a series of potent and selective small molecule RPA inhibitors to address the underlying mechanism of DDR inhibition and RPA exhaustion. We have optimized the NERx 551 inhibitors via separation and assessment of two active enantiomers that reveal two unique orientations that inhibit RPA binding to DNA. Chemical optimization for solubility, cellular uptake and bioavailability has generated a candidate drug with excellent characteristics that significantly enhance cellular activity. Our cellular and *in vivo* data demonstrate a role for RPA inhibition in combination therapy with DNA damaging agents that suggest a global mechanism of action. Data suggest that small molecule inhibition of RPA-DNA binding mimics RPA exhaustion and can be exploited as a single agent and in combination therapy with both DDR targeted agents and common DNA damaging chemotherapeutics.

Supported by NIH Grant R01CA180710 and the Tom and Julie Wood Family Foundation.

Basic Science ***Research Technician***

POSTER #61

TARGETING MEMBRANE HYPEREXCITABILITY OF DORSAL ROOT GANGLION NEURONS TO TREAT NEUROPATHIC PAIN ASSOCIATED WITH OXALIPLATIN

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Objectives: Chemotherapy-induced peripheral neuropathy (CIPN) is a dose-limiting neurotoxicity caused by chemotherapy drugs. CIPN is frequently associated with neuropathic pain that significantly impairs the quality of life and work capability of cancer patients. Use of oxaliplatin for colorectal cancer is associated with a characteristic cold-sensitive pain in addition to mechanical allodynia. The objective of this work was to study the effects of membrane excitability suppression in peripheral sensory neurons located at dorsal root ganglion (DRG) on thermal and mechanical sensitivity caused by oxaliplatin.

Methods: Membrane excitability were recorded by whole-cell patch clamping in DRG neurons dissociated from adult C57BL/6 mice. Pain and motor behaviors were examined in mice treated with oxaliplatin. Cold, heat, mechanical sensitivity, and motor function were assessed by acetone spray, Hargreaves, Von-Frey, and Rotarod tests, respectively.

Results: In small DRG neurons, oxaliplatin induced membrane hyperexcitability including membrane depolarization, reduced threshold for action potentials, and increased firing numbers of action potentials. The oxaliplatin-induced membrane hyperexcitability was completely reversed by retigabine, a FDA approved anti-epileptic drug and a KCNQ/M channel opener. In vivo, oxaliplatin induced cold and mechanical hypersensitivity while it did not change heat sensitivity or motor function. Retigabine inhibited oxaliplatin-induced cold and mechanical hypersensitivity without changing heat sensitivity or motor function.

Conclusions: Membrane hyperexcitability caused by membrane depolarization in nociceptive DRG neurons contribute to neuropathic pain associated with oxaliplatin. Retigabine inhibits oxaliplatin-induced neuropathic pain by hyperpolarization of membrane potential of nociceptive DRG neurons. Targeting membrane hyperexcitability is a useful strategy to treat neuropathic pain associated with oxaliplatin.

(Zhiyong Tan was supported by an ACS-IUSCC pilot fund IRG-84-002-28 and a Showalter Research grant)

Basic Science ***Visiting Research Associate***

POSTER #62

TARGETING DNA REPAIR AND DNA DAMAGE RESPONSE (DDR) FOR CANCER THERAPY

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Targeting DNA repair and the DNA damage response (DDR) for cancer therapy has recently gained increasing attention with inhibitors of the PARP enzyme showing a therapeutic efficacy in various cancers. Solid tumors of the lung, pancreas, breast, and ovary represent a continuing clinical challenge in treatment and together account for over 250,000 deaths in the US alone, representing over 40% of all cancer deaths. There are limited therapeutic options for many of these patients, and molecularly targeted and combination therapies remain necessary for treating these aggressive cancers. The opportunity exists to exploit recent scientific advances in our knowledge of the underlying biology behind these cancers to create novel targeted therapeutics to dramatically enhance patient response to therapy and ultimately survival. It is well understood that various cancer treatments like cisplatin, etoposide and ionizing radiation (IR) impart their chemotherapeutic effect by the formation of direct DNA damage which block DNA replication and transcription culminating in apoptosis. It is also well established that repair of this DNA damage by non-homologous end joining (NHEJ) or nucleotide excision repair (NER) reduces the effectiveness of radio- or chemo-therapy. In continuation of our research in the DNA repair field, we have developed a series of novel small chemical molecules that disrupt critical protein-DNA interactions by targeting NHEJ and NER pathways individually. We will discuss the **discovery and development of highly potent and selective DNA-PK inhibitors**: The DNA dependent protein kinase (DNA-PK) is a validated target for cancer therapeuticsthat drives the DDR and plays a necessary role in the NHEJ DSBs repair pathway. To date, development of inhibitors for DNA-PK has focused on targeting the active site with ATP mimetics. We have taken the novel and unique approach to inhibiting DNA-PK via blocking the Ku70/80 heterodimer interaction with DNA, an essential step in DNA-PK activation. Exploiting this unique mechanism of kinase activation, we have identified a series of potent and specific DNA-PK inhibitors that impart their inhibitory activity via disruption of the binding of Ku protein to DNA ends.

We will also present data on the development of Replication Protein A (RPA)-DNA interaction inhibitors: RPA plays a crucial role in the NER pathway and the DDR and thus is a novel drug target to develop novel cancer therapy. The series of novel small molecule inhibitors that we have developed targeting RPA independently exhibit single-agent anti-cancer activity in several cancer cell lines, potentiate cellular sensitivity to chemotherapeutic agent and showed synergy with Pt in xenograft mice model.

In summary, our *in vitro* and cellular data of both projects demonstrate that these small molecule inhibitors can be further developed as anti-cancer therapeutics that can be used as an adjuvant to, or concomitant with DNA damaging agents and radiotherapy.

Basic Science

POSTER #63

KUB5-HERA DEFICIENCY PROMOTES "BRCANESS" AND VULNERABILITY TO PARP INHIBITION IN BRCA-PROFICIENT BREAST CANCERS

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Purpose: Clinical trials of Poly(ADP-ribose) polymerase (PARP) inhibitors surprisingly show positive clinical benefits in patients without *BRCA* mutations (germline or somatic). This finding clearly posits a search for novel biomarkers and innovative strategies to expand the use of PARP inhibitors beyond BRCA deficiency. Here, we investigated the functional role of Kub5-Hera^{RPRD1B} in homologous recombination (HR) repair and its potential clinical significance in targeted cancer therapy.

Experimental Design: Functional characterization of K-H alterations on HR repair of double-strand breaks (DSB) were assessed by targeted gene silencing, plasmid reporter assays, immunofluorescence and Western blots. Cell survival with PARP inhibitors was evaluated through colony forming assays and statistically analyzed for correlation with K-H expression in various *BRCA1/2* nonmutated breast cancers. Gene expression microarray/qPCR analyses, chromatin immunoprecipitation, and rescue experiments were used to investigate molecular mechanisms of action.

Results: K-H mediates homologous recombination (HR) by facilitating the recruitment of RNAPII to the promoter region of a critical DNA damage response and repair effector, cyclin-dependent kinase 1 (*CDK1*) – crucial for BRCA1 activation (pS1497). K-H loss in BRCA-proficient cells promotes BRCAness, which consequently relies on extensive poly(ADP-ribose) polymerase 1 (PARP1) activity for survival. Indeed, PARP inhibition in K-H deficient cells led to synthetic lethality.

Conclusions: Cancer cells with K-H deficiency have exploitable BRCAness properties that greatly expands use of PARP inhibitors beyond BRCA mutations. Our results suggest that aberrant K-H alterations have vital translational implications in cellular responses/survival to DNA damage, carcinogenesis and personalized medicine.

Basic Science

POSTER #64

FACTORS ASSOCIATED WITH ADHERENCE TO MAMMOGRAPHY SCREENING AMONG INSURED WOMEN; DO THE FACTORS DIFFER BY INCOME LEVEL?

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Background: Breast cancer is the second leading cause of cancer mortality; however, mammography screening rates remain less than optimal. Multiple factors contribute to non-adherence, including income. The purpose of this study was to compare factors, including concepts from the Health Beliefs Model, predicting mammography adherence across income groups.

Methods: Women (n=1,681) with health insurance, and with no mammogram in the last 15 months were enrolled to participate in an interventional study. Binary logistic regression was used to estimate multivariable-adjusted odds ratios for demographic and health belief factors predicting mammography adherence for each income group: 1) low <$30,000, 2) middle $30,000-$75,000 and 3) high >$75,000.

Results: Being in the contemplation stage (compared to pre-contemplation) of obtaining a mammogram predicted mammography adherence across all income groups and was the only predictor in the middle-income group (OR=3.9, 95% CI: 2.61-5.89). Increase in age was associated with 5% increase in mammography adherence for low (OR=1.05, 95% 1.01-1.09) and high-income (OR=1.05, 95% CI: 1.02-1.08) women. Having a doctor recommendation predicted mammography adherence only in low-income women (OR=10.6, 95% CI: 2.33-48.26), while an increase in perceived barriers inversely predicted mammography adherence only among high-income women (OR=0.96, 95% CI: 0.94-0.99). In post-hoc analysis, the two most frequent barriers reported by high-income women were difficulty in remembering appointment (53%) and lack of time to get mammogram (24%).

Conclusions: Factors contributing to mammography adherence differed by income groups in insured women. Future interventions to increase mammography adherence must consider socioeconomic factors and be tailored to meet the needs of a diverse sample.

Behavioral ***Faculty***

POSTER #65

MEASUREMENTS OF TELOMERE LENGTH AND MTDNA COPY NUMBER BEFORE-AFTER ACCEPTANCE AND COMMITMENT THERAPY FOR CANCER SURVIVORS WITH FEAR OF RECURRENCE

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Fear of cancer recurrence (FCR) is one of the most prevalent, persistent, and disruptive sources of distress for adult cancer survivors. After completing treatment, 44-56% of survivors continue to report clinically significant FCR well into disease-free survivorship. FCR is associated with maladaptive coping and reduced quality of life. Despite being the most frequently identified unmet supportive care need reported by survivors, few empirically supported treatments exist for FCR. Telomeres exist on the ends of chromosomes to protect and maintain their function. It has been shown a strong link between life stress and telomere shortening has been drawn that implicatesthe psychological effect on molecular changes in the body. Additionally, mitochondrial DNA (mtDNA), located close to the source of reactive oxidative stress production, is extremely susceptible to oxidative stress and aging. Therefore, we hypothesized that telomere lengths and mtDNA copy number levels could be altered between different psychological interventions. To address this hypothesis, we placed breast cancer survivors into three intervention therapy groups and telomere lengths as well as mtDNA copy number levels were measured using their white blood cells at the following timepoints: baseline (T1), postintervention (T2), 1month followup (T3), and 6month followup (T4). Total seventysix breast cancer survivors were split into the following groups: Acceptance and Commitment Therapy (ACT), Survivorship Education (SE) intervention, and Enhanced Usual Care (EUC). The ACT and SE interventions were delivered in a group format 2 hours weekly for 6 weeks. EUC was largely self-administered with a packet of readings on coping with FCR and other common sources of distress during post-treatment survivorship. The preliminary results indicate that the ACT therapy may have a positive impact on telomere lengths and mtDNA copy number levels. We are currently analyzing the data by adjusting with several variables including age, BMI, stage of breast cancer, and time since diagnosis. We will discuss the data for details in the poster.

Behavioral ***Graduate Student***

POSTER #66

PRE-TREATMENT SYMPTOM CLUSTERS AND THEIR ASSOCIATION WITH LONGITUDINAL COGNITIVE FUNCTION IN OLDER BREAST CANCER SURVIVORS

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Background: Symptom clusters can affect cancer survivors' functional outcomes. Older survivors may be especially vulnerable to functional decline but there is little research on symptoms and cognition in older survivors.

Methods: Survivors with newly diagnosed non-metastatic breast cancer (n=320) ages 60+ without dementia or neurological disease were recruited at six US sites from August 2010-December 2015. Participants completed surveys and neuro-psychological tests before systemic therapy, and 12 and 24 months later. Latent class analysis was used to identify symptom clusters based on baseline self-reported depression, anxiety, fatigue, sleep disturbance, and pain. The FACT-Cog measured self-reported cognition. Standardized neuropsychological tests measured cognitive function in two domains: attention, processing speed, and executive function [APE] and learning and memory [LM]). Linear mixed-effects models examined the effects of symptom-cluster group on cognitive scores over time, controlling for age, WRAT, race, site, stage, and comorbidity.

Results: Survivors ranged from 60-98 years of age (mean 68), 79% were White, most were well educated, and 67% had DCIS or Stage 1 cancers. Two non-overlapping symptom clusters were present: high symptom burden (n=48; 15%) and low symptom burden (n=272; 85%). Compared to the low symptom group, survivors with high burden had lower adjusted APE across all time points (Est [SE]=-0.20[.10], P=0.04). The high burden group had lower adjusted baseline LM (-0.34[.13], P=0.009) and more self-reported cognitive problems (-16.28[3.13], P<0.0001); over time, scores tended to continue to be lower for the high vs. low burden group.

Conclusions: Only a small proportion of older breast cancer survivors have a high symptom burden at diagnosis, but they have meaningful decrements in cognition. Symptom burden prior to systemic therapy may be a risk-marker for lower cognitive function. If confirmed, symptom management may improve cognitive and other survivorship outcomes. Biological pathways related to symptoms and cognitive decline may suggest shared mechanisms.

Behavioral ***Graduate Student***

POSTER #67

ANALYSIS OF RETROSPECTIVE VS PROSPECTIVE PEER REVIEW IN A MULTISITE ACADEMIC RADIATION DEPARTMENT

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Purpose/Objectives

Our multisite academic radiation department transitioned from weekly retrospective to daily prospective peer review to improve plan quality and decrease the rate of plan revisions after treatment start. We review our initial experience regarding deviation patterns and time from simulation to treatment start.

Materials/Methods

In all, 798 patients with 1124 plans were reviewed: 611 plans weekly from 7/12 to 10/18/17 and 513 plans daily from 10/16/17 to 1/12/18. In the weekly era, plan review primarily occurred after treatment start (5.6% prospectively reviewed) and was based on screenshots of various plan aspects. In the daily era, plan review was performed in ECLIPSE with emphasis on prospective timing of review (75.4% prospectively reviewed).

Plans were assessed for appropriateness of treatment intent, dose-fractionation, modality, contours, target coverage, and risk to critical structures. Deviations were major if plan revisions were recommended prior to the next fraction and minor if modifications were suggested but not required for that course. All physicians and representatives from dosimetry and physics were required to attend. Categorical variables were compared using Chi-squared tests of independence; means were compared using independent t-tests.

Results

Overall, 76 (6.8%; N = 31 major) deviations were noted. Rates of any deviation were increased in the daily era (8.6% vs 5.2%, $p = 0.031$) and with prospective review (9.7% vs 5.0%, $p = 0.003$), with higher rates of major deviations in the daily era (4.1% vs 1.6%, $p = 0.016$ major; $p = 0.542$ minor) and with prospective review (5.0% vs 1.4%, $p = 0.001$ major; $p = 0.347$ minor).

In the subset of plans with simulation followed by treatment start within 1-2 weeks (N = 844), mean working days between simulation and treatment was similar across eras (mean = 5.55 days vs 5.53 days, $p = 0.923$) but was increased with prospective review (mean = 6.04 days vs 5.22 days, $p = 0.001$).

Recommendations for plan revision due to any deviation (N = 76) were incorporated at a higher rate in the daily era (84.1% vs 31.3%, $p < 0.001$). The rate of plan revisions after treatment start due to peer review was decreased with prospective review (7.3% vs 28.6%, $p = 0.030$). The rate of recommendation for plan revisions after treatment start was decreased in the daily era (15.9% vs 40.6%, $p = 0.02$) and with prospective review (12.2% vs 42.9%, $p = 0.004$).

Conclusions

Daily peer review with emphasis on prospective plan evaluation was related to increased rates of deviations recorded and corrected without a prolonged interval between simulation and treatment. Daily prospective plan review is feasible in a multisite academic setting and improves plan quality without delaying patient care.

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

POSTER #68

FEASIBILITY, ACCEPTABILITY, AND PRELIMINARY EFFECTS OF A MINDFULNESS MEDITATION INTERVENTION FOR ADULTS WITH ADVANCED CANCER AND FAMILY CAREGIVERS: A RANDOMIZED PILOT

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Background: Patients with advanced cancer often avoid emotionally-sensitive discussions with family caregivers (FCGs) about their end-of-life (EOL) treatment preferences. Avoiding these discussions inhibits appropriate EOL preparations and may reduce quality of life (QoL) for patients and their FCGs. Mindfulness training facilitates emotional regulation, adaptive coping, and strengthened communication skills and was tested in this randomized pilot.

Methods: Eligible patients had a: (1) locally-advanced solid malignancy; (2) life expectancy < 12 months as rated by their oncologist; (3) score of = 7 on cancer-related cognitive avoidance (Mini-MAC); and (4) FCG willing to enroll. Patient-FCG dyads (n = 55) were randomly assigned to a 6-session mindfulness meditation class with communication training or usual care. Primary endpoints were feasibility, retention, QoL, and intervention acceptability for dyads randomized to mindfulness (assessed by attendance and reported home practice of mindfulness). Outcomes were assessed at baseline and 6- and 10-weeks using intent-to-treat analysis.

Results: Of 133 patients who screened eligible, 41% enrolled. Most patients (85%) had stage IV cancer, with breast (29%) and GI (27%) cancers being most prevalent. Dyadic retention was 84% through 10 weeks. Intervention acceptability data from mindfulness dyads show 71% of patients and 74% of FCGs felt satisfied with the intervention, with 76% of mindfulness dyads indicating they would highly recommend the study practices to others. Further, 88% of mindfulness patients and 70% of mindfulness FCGs continued their informal mindfulness practice at least three days a week after the commencement of the class. Participant attendance averaged at 4.2 out of 6 sessions (SD=2.2), with 70% of dyads attending 5 or 6 sessions. Participants in the intent-to-treat sample engaged in home practice an average of 3.7 days per week. Overall 83.3% of all mindfulness participants reported any home practice, averaging 4.7 days per week.

Mindfulness patients also reported a large and significant improvement in existential QoL ($d = 0.82$, $p = 0.009$) at 6 weeks compared to controls; however, the magnitude of improvement was not sustained at 10 weeks ($d = 0.24$, $p = 0.43$). Mindfulness FCGs reported a significant *within-group* improvement in QoL at 10 weeks ($d = 0.45$, $p = 0.03$); however, *between-group* comparisons were not significant at any time point.

Conclusions: The trial was feasible and mindfulness was acceptable to dyads randomized to the intervention. Within limits of a small pilot, results suggest that mindfulness training is potentially beneficial

for improving QoL in dyads coping with advanced cancer. A full-scale efficacy trial is planned.

Behavioral Research Technician

POSTER #69

NOVEL USE OF CO-DESIGN TO CREATE A HAND HYGIENE VIDEO FOR PEDIATRIC ONCOLOGY POPULATION

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While the survival rate of the pediatric oncology population has increased, infections are a leading cause of morbidity and mortality. There are inconsistent recommendations in non-pharmacological methods to decrease chances of acquiring infections. A quality improvement project was funded by the Women for Riley to investigate current infection prevention recommendations by using co-design to create an educational video for patients and their caregivers.

The co-design process is a unique approach to design and production that involves engagement and activation from all stakeholders. Patient and caregiver experiences are traditionally captured through satisfaction surveys, complaints, comment cards and interviews, “yet these tools tend to simply ask how people rate their experience rather than fully capturing the experience and gathering ideas for how it could be improved” (Ruth Tollyfield, 2014). Engaging in issues that matter to patients increases the likelihood that research will be relevant to patients’ lives; “vital to co-created research is arriving at a common understanding of ‘the problem’ and developing a path toward the ‘solution’” (Simpson & Seibold, 2008). Since children have very different perceptions of the world and express their thoughts differently than adults, an educational video should focus on what the patients identify as their greatest needs to help them cope with their cancer treatment (Ruland, Starren, & Vatne, 2007). With the use of co-design, partnerships between patients, caregivers and medical staff that may lead to deeper, long-term changes in attitudes and behaviors that can improve the quality of not only patient education, but medical care (Glenn Robert et al., 2015).

The project consisted of literature review, patient/caregiver interviews, clinical staff questionnaire and patient/caregiver workshops. Based upon the information gathered in a co-design process, the topic of hand hygiene education was selected. Every patient/caregiver dyad stated that handwashing was the most effective infection prevention method. However, during the patient workshop in which seven patients and some of their family members participated, it was revealed that patients and caregivers did not correctly wash their hands. Therefore, an educational video was created based upon input from patients, caregivers, oncology clinicians, nursing, infection disease staff, child life specialists, hospital administration, hospital marketing and the Women for Riley. The completed video can be accessible at home, at the hospital while inpatient, and in the Hematology/Oncology outpatient clinic. We were successful in using the co-design process to create a well-received educational video on hand hygiene that filled a knowledge gap on infection prevention for our patient population.

Population Science/Epidemiology ***Clinical Nurse***

POSTER #70

A STRATIFIED GEOGRAPHIC SURVEY SAMPLE OF THE INDIANA UNIVERSITY CANCER CENTER CATCHMENT AREA

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The Hoosier Health survey assessed knowledge, beliefs, and behaviors regarding cancer screening and prevention strategies, as well as information-seeking behaviors and preferences among adults in the Indiana University Cancer Center catchment area.

A survey was mailed to a sample of 8,000 individuals. Eligible participants were 18-75 years old, White/Caucasian or Black/African-American, had visited a statewide academic integrated delivery system in the 12 months prior to sampling, and resided in the 34 counties with the highest cancer mortality rates. Because electronic medical record data contained mailing address but not county of residence, data were first pulled for all eligible patients in zip-codes that intersected with 34 counties of interest, and were then geocoded to identify and select for county of residence.

The sample was stratified by location (Urban, Rural) and race (White, Black), with a goal of 2000 individuals sampled in each of the four strata (Urban/Black, Urban/White, Rural/Black, Rural/White). Urban/Rural location was identified from census-tract according to Rural-Urban Commuting Areas (RUCA) codes[1],[2],[3],[4]. Because there were less than 2,000 total individuals in the sampling frame for the Rural/Black strata, the remainder of the 4,000 individuals in the Rural areas were sampled from the Rural/White category to ensure 4,000 Rural and 4,000 Urban respondents for the primary comparisons between these geographic areas.

The sampling frame of 284,062 total eligible participants was further divided into 16 sub-strata based on location, race, sex, and age group (18-49, 50-75 years). The 8,000 individuals for mailing were randomly sampled from the 16 sub-strata with an additional implicit stratification by census tract to further distribute units across geographic areas.

This provided a sample of 4,000 individuals from Urban areas and 4,000 individuals from Rural areas. Within the Urban group, the sample included 2,000 Black and 2,000 White. Within the Rural group, only 468 were available to select from the Black group, so 3,532 were sampled from the White group, to maintain 4,000 total Rural. Survey weights were created for use in analysis, which account for the probability of selection within each strata.

Surveys returned from 739 participants (9.2%) were available for interim analysis, while an additional 450 are expected from a second mailing. Current response rates are highest among White populations (10% Rural, 11% Urban) while Black/African American responses were lower (7% Rural, 6% Urban).

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POSTER #71

PREVALENCE OF ADVANCED, PRECANCEROUS COLORECTAL NEOPLASIA IN BLACK AND WHITE POPULATIONS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: Colorectal cancer (CRC) incidence and mortality is higher in Blacks than in Whites. While the reason(s) for these disparities is unclear, some guidelines recommend CRC screening in Blacks starting at age 40-45.
Objective: To compare the prevalence of advanced adenoma (AA) or advanced, precancerous colorectal neoplasia (ACN) between asymptomatic Black and White screen-eligible adults.
Methods: We performed a systematic review and meta-analysis by first searching OvidMEDLINE, PubMed, EMBASE, and the Cochrane Library to identify published literature from database inception to June 2017. We included studies measuring the prevalence of AA or ACN in average-risk Black and White persons undergoing screening colonoscopy. Two authors independently assessed study quality and risk for bias using a modified NIH quality assessment instrument for cross-sectional studies, and independently abstracted descriptive and quantitative data from each study. A random effects model for meta-analysis was used, providing the I^2 measure for heterogeneity, risk differences (RD) and odds ratios (OR).
Results: From 1653 titles, we identified 9 studies that included 302,128 subjects. The largest single study included 292,494 (97%) of all subjects. Six of 9 studies were of high methodological quality and low risk for bias. Overall prevalence of AA/ACN was no different between Blacks and Whites: OR=1.03; 95% CI, 0.81-1.30 and RD=0.00; 95% CI, -0.01 to 0.02; I^2=52% showing moderate heterogeneity. Proximal AA/ACN prevalence was greater in Blacks than in Whites: OR=1.20; CI, 1.12-1.30 and RD=0.01; CI, 0.00 to 0.01; I^2=0 showing low heterogeneity. Excluding the largest study resulted in no difference in the prevalence of overall AA/ACN (OR=0.99; CI, 0.73-1.34) or proximal AA/ACN (OR=1.48; CI, 0.87-2.52). Including only the highest quality studies for which pathology was available (study N = 5, subject N = 8,503) showed no difference in AA/ACN prevalence (OR=1.06; CI, 0.75-1.50) or proximal AA/ACN prevalence (study N = 3, subject N = 7,187): OR=1.44; CI, 0.84-2.49.
Conclusion: Prevalence of AA/ACN in similar in average-risk Black and White screen-eligible persons, findings that support CRC screening beginning at age 50, irrespective of race

Population Science/Epidemiology ***Faculty***

POSTER #72

ASSOCIATIONS BETWEEN INTAKE OF CALCIUM, MAGNESIUM, AND PHOSPHORUS AND RISK OF PANCREATIC CANCER IN A POPULATION-BASED CASE-CONTROL STUDY IN MINNESOTA

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Pancreatic cancer is the fourth leading cause of cancer death among both men and women in the U.S. and has a dismal prognosis among all cancers due to its aggressive nature and lack of effective screening tests. Pancreatic cancer etiology remains elusive, with few well-established risk factors aside from cigarette smoking; therefore, it is critical to identify other modifiable risk factors for primary prevention. Calcium, magnesium, and phosphorus are essential minerals for bone health and other important metabolic processes. Emerging evidence indicates that these minerals are involved in the carcinogenesis of the colon and other organs through their influence on cellular proliferation, systematic inflammation, immune functions, and/or genomic stability. In the present study, we aimed to investigate the associations between intake of calcium, magnesium, and phosphorus and risk of pancreatic cancer in a case-control study conducted during 1994-1998 in Minnesota. Cases (n=150), aged 20 years or older, were ascertained from all hospitals in the metropolitan area of the Twin Cities and the Mayo Clinic; from the latter, only cases residing in the Upper Midwest of the US were recruited. Controls (n=459) were randomly selected from the general population and frequency matched to cases by age (within 5
years) and sex. Dietary and supplemental intake of three minerals of interest and other nutrients was estimated from a validated food frequency questionnaire. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated using unconditional logistic regression. After adjustment for age, sex, race, education, physical activity, cigarette smoking, alcohol use, and intake of energy, total fat, fiber, fruits, and vegetables, risk of pancreatic cancer decreased with an increasing dietary intake of calcium [OR (95% CI) for quartiles 2, 3, and 4 vs. quartile 1: 0.96 (0.54-1.67), 0.65 (0.35-1.17), and 0.58 (0.23-1.40), p-trend = 0.09]. A similar inverse but not significant association was observed for total calcium intake. A significantly reduced risk of pancreatic cancer was found among subjects in the third quartile of total magnesium intake compared with those in the first (lowest) quartile [OR (95% CI): 0.35 (0.17-0.69)], although it may be a chance finding due to multiple comparisons. No apparent associations existed between dietary and total intake of phosphorus and risk of pancreatic cancer. Our study shows that there are suggestive, but inconsistent beneficial effects of dietary and/or supplemental intake of calcium and magnesium on the occurrence of pancreatic cancer. This observation warrants confirmation in other epidemiologic studies.

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

POSTER #73

THE RELATIONSHIP BETWEEN MAMMOGRAPHY ADHERENCE AND A MULTI-FACTOR LIFESTYLE INDEX

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Purpose/Background: The purpose of our study was to determine whether multi-lifestyle factors predict mammography screening in U.S. women. It is well established that several lifestyle factors, including physical activity, alcohol intake, smoking, BMI, and dietary factors, influence breast cancer risk. However, the link between these factors and mammography screening remain unclear. Moreover, a multi-factor lifestyle approach may be more informative than looking at single individual factors.

Methods: Women aged 50-75 years who were non-adherent to breast cancer screening guidelines (n=858) were enrolled in an intervention study to promote adherence. Medical records were obtained at 6 months post intervention to verify screening. Women were surveyed at baseline and provided information on their vegetable intake, physical activity, smoking, BMI, and alcohol intake in order to create a combined multi-factor lifestyle index. The index scores were based on CDC guidelines, and the scores ranged from 0-20. Binary logistic regression was used to estimate multivariable-adjusted odds ratios (OR) and 95% confidence intervals (CI).

Results: The mean score for the multi-factor index was 11.54. Overall, an increase in theindex score predicted mammography adherence [OR = 1.05 (1.00 – 1.10)]. However, the association was much stronger among subjects who received a doctor's recommendation for mammography [OR = 1.10 (1.04 – 1.18)], while no association was observed among those without a doctor's recommendation [OR = 0.97 (0.90 – 1.06)]. When assessing the association of single lifestyle factors, non-smoking [OR = 1.12 (1.00 – 1.26)] and high physical activity [OR = 1.16 (1.04 – 1.31)] significantly predicted mammography screening. Other lifestyle factors were not associated with mammography adherence.

Conclusion: Women who engaged in a healthier behavioral lifestyle were more likely to be screened for breast cancer, especially if they received a physician recommendation for breast cancer screening. These findings suggest that encouraging a healthier lifestyle and increasing patient-physician communication regarding breast cancer screening may increase mammography adherence among U.S. women.

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

POSTER #74

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

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Background: Mitochondrial DNA (mtDNA) is particularly vulnerable to oxidative stress and mutations. As a promising biomarker of oxidative stress-related health outcomes, mtDNA copy number (mtCN) in peripheral blood leukocytes has been associated with a range of diseases, including cancers. Prospective studies on the association between mtCN and colorectal cancer (CRC) risk are limited and findings are inconsistent.

Method: We examined the association between pre-diagnostic mtCN and CRC risk in two prospective case-control studies nested within the Nurses' Health Study (NHS, all women, 324 cases and 677 controls) and the Health Professional Follow-Up Study (HPFS, all men, 289 cases and 587 controls). Cases and controls are matched by age, race, fasting status, and time of blood draw. Relative mtCN in peripheral blood leukocytes was measured by quantitative PCR-based assay. Conditional logistical regression models adjusting for confounding variables were employed to estimate odds ratios (ORs) and 95% confidence intervals (95% CIs) for the association between log-transformed mtCN (log-mtCN) and CRC risk in women and men.

Results: Higher log-mtCN levels were significantly associated with lower risk of colorectal cancer among women; compared to the lowest quartile, the multivariable-adjusted ORs (95% CIs) for 2nd quartile, 3rd quartile, and 4th quartile were 0.56 (0.36-0.89), 0.51 (0.32-0.82), and 0.33 (0.20-0.55), respectively (*P* for trend < 0.0001). However, the association between log-mtCN and CRC risk was not statistically significant among men; the multivariable-adjusted ORs (95% CIs) for 2nd quartile, 3rd quartile, and 4th quartile were 1.08 (0.66-1.77), 0.82 (0.49-1.39), and 0.86 (0.52-1.43), respectively, compared to the lowest quartile (*P* for trend = 0.40).

Conclusion: Our study showed significant inverse association of pre-diagnostic leukocyte mtCN and CRC risk in women but not in men.

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

POSTER #75

MODERN RADIATION FURTHER IMPROVES SURVIVAL IN NON-SMALL CELL LUNG CANCER: AN ANALYSIS OF 288,670 PATIENTS

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Introduction: Radiation therapy plays an increasingly important role in the treatment of patients with non-small-cell lung cancer (NSCLC). The purpose of the present study is to assess the survival outcomes of radiotherapy treatment compared to other treatment modalities and to determine the potential role of advanced technologies in radiotherapy on improving survival.

Methods: We used cancer incidence and survival data from the Surveillance, Epidemiology, and End Results database linked to U.S. Census data to compare survival outcomes of 288,670 patients with stage I-IV NSCLC treated between 1999 and 2008. The primary endpoint was overall survival.

Results: Among the 288,670 patients diagnosed with stage I-IV NSCLC, 92,374 (32%) patients received radiotherapy—almost double the number receiving surgery (51,961, 18%). Compared to other treatment groups and across all stages of NSCLC, patients treated with radiotherapy showed greater median and overall survival than patients without radiation treatment ($p < 0.0001$). Radiotherapy had effectively improved overall survival regardless of age, gender, and histological categorization. Radiotherapy treatment received during the recent time period 2004 – 2008 is correlated with enhanced survival compared to the earlier time period 1999 – 2003.

Conclusion: Radiation therapy was correlated with increased overall survival for all patients with primary NSCLC across stages. Combined surgery and radiotherapy treatment also correlates with improved survival, signaling the value of bimodal or multimodal treatments. Population-based increases in overall survival were seen in the recent time period, suggesting the potential role of advanced radiotherapeutic technologies in enhancing survival outcomes for lung cancer patients.

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

POSTER #76

A PLASMA TELOMERIC CELL-FREE DNA LEVEL IN UNAFFECTED WOMEN WITH BRCA1 AND/OR BRCA2 MUTATIONS

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Excessive telomere shortening is observed even in non-invasive breast cancer lesions when compared to adjacent non-cancerous tissues, suggesting that telomere length may serve as a key biomarker for early cancer detection.Plasma cell-free DNA (cfDNA) is a small DNA fragment circulating in the bloodstream originating from both non-tumor- and tumor-derived cells. We have found that a plasma telomeric cfDNA level decreases in sporadic breast cancer patients compared to controls. Because tumor suppressor gene products including BRCA1 and BRCA2 (BRCA1&2) play an important role in telomere maintenance, we hypothesized that the plasma telomeric cfDNA level is associated with the mutation status of BRCA1&2 genes. To test this hypothesis, we performed plasma telomeric cfDNA quantitative PCR (qPCR)-based assays to compare 28 women carriers of the BRCA1&2 mutation with age-matched controls of 28 healthy women. The results showed that the plasma telomeric cfDNA level was lower in unaffected BRCA1&2 mutation carriers than in age-matched controls from non-obese women (BMI < 30), while there was no association between unaffected BRCA1&2 mutation carriers and age-matched controls in obese women (BMI > 30). Moreover, unlike leukocyte telomere length, the plasma telomeric cfDNA level applied aptly to the Tyrer-Cuzick model in non-obese women. These findings suggest that circulating cfDNA may detect dysfunctional telomeres derived from cells with BRCA1&2 mutations and therefore its level is associated with breast cancer susceptibility. This pilot study warrants further investigation to elucidate the implication of plasma telomeric cfDNA levels in relation to cancer and obesity.

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

POSTER #77

PREVALENCE AND TRENDS OF OBESITY AND METABOLIC SYNDROME AMONG U.S. CANCER SURVIVORS COMPARED TO ADULTS WITH NO CANCER, THE NHANES 1999-2014

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Background: The prevalence of obesity and Metabolic syndrome (MetS) in the general population has been documented extensively. While obesity and MetS are associated with cancer risk and may persist after diagnosis, the prevalence and the secular trends of obesity and MetS among cancer survivors has not been examined in a nationally representative cohort.

Objective: To compare adults diagnosed with cancer and those without cancer on the prevalence of obesity and MetS in 2013-2014, and on the time trends over the past years from 1999 through 2014.

Methods: A sample of 39,608 adults, aged 20+ who participated in National Health and Nutrition Examination Survey (NHANES) surveys from 1999-2014 were included in the study. NHANES is a cross-sectional, nationally representative survey of non-institutionalized U.S. civilians. Of those surveyed, 2,811 subjects had been diagnosed with non-skin cancers, and 1,770 with obesity-linked cancers (colorectal, liver, kidney, esophageal, stomach, pancreatic, gallbladder, breast, endometrial, ovarian and prostate). MetS was defined as having at least three of the following five factors: (1) high-density lipoprotein (HDL) cholesterol (<50 mg/dL women and <40mg/dL men) or use of cholesterol lowering medication, (2) serum triglyceride level (>150 mg/dL), (3) systolic (>130 mm Hg) or diastolic blood pressure (>85 mm Hg), or use of anti-hypertensive medication, (4) blood glucose (>100 mg/dL) or self-reported diabetes or use of insulin, (5) waist circumference (>88 cm women and >102 cm men).

Results: In 2013-2014, the prevalence of obesity was 41.1%, 40.5% and 37.8% among adults diagnosed with obesity-linked cancers, total-cancer, and no cancer diagnosis, respectively. Trends in the prevalence of obesity have significantly increased over the last 16 years for all three groups. Relative to adults without a cancer diagnosis, the trend for obesity prevalence was significantly higher for those with obesity-linked cancers (p=0.019), but not with total cancer (p=0.35). In relation to MetS, the 2013-2014 prevalence was significantly higher among adults with obesity-linked cancers (52.7%) and total cancer (50.3%) compared to adults without cancer (29.2%). Over the 16-year period from 1999-2014, the prevalence of MetS has increased among adults with obesity-linked cancers (p=0.008), total cancer (p=<.0001), and those without a cancer diagnosis (p=0.005), respectively. Compared to the adults without a cancer diagnosis, the prevalence of MetS over the years has significantly increased among adults with obesity-linked cancers (p=0.004) and total cancer (p=0.05) were significantly higher.

Conclusion: U.S. adults diagnosed with obesity-linked cancers had a higher prevalence of obesity and MetS, and the prevalence have significantly increased over the last 16 years, compared to those without a cancer diagnosis. The findings of this study underscore the urgent need for clinicians and public health workers to recognize and address cardiometabolic health problems among cancer survivors.

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

POSTER #78

CIGARETTE PRICE, SMOKING BEHAVIORS, AND LUNG CANCER MORTALITY IN INDIANA

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Introduction: Increasing tobacco costs have been proven to be one of the most effective interventions of decreasing tobacco use. The relationship between tobacco cost and lung cancer mortality has not been as well established. We investigated the relationship of cigarette price with smoking prevalence, cigarette consumption, and lung cancer incidence and mortality in Indiana and nationally.

Methods: We obtained average cigarette pack prices, cigarette pack sales, smoking prevalence, and lung cancer incidence and mortality rates in Indiana and nationally from 1995-2015. Average cigarette pack prices were inflation adjusted to 2015 then assessed for Pearson correlation coefficient (r) with cigarette pack sales, smoking prevalence, and lung cancer incidence and mortality. Cigarette price was also correlated with smoking prevalence among state-level characteristics that included gender, age, ethnicity, education, and income.

Results: From 1995 to 2015, average cigarette pack price in Indiana rose from $2.29 to $5.41. Increasing cigarette price in Indiana was associated with decreasing cigarette consumption (r= -0.91, $p<0.001$) and decreasing overall smoking prevalence (r= -0.72, $p<0.001$). However, those in the lowest income level had higher smoking prevalence associated with rising cigarette price (r= 0.67, $p=0.001$). Increasing cigarette price correlated with decreasing lung cancer mortality both in Indiana (r= -0.79, $p<0.001$) and nationally (r= 0.96, $p<0.001$).

Conclusions: Increasing tobacco taxes and subsequent increasing cigarette prices were associated with decreased smoking prevalence, cigarette consumption, and lung cancer mortality in Indiana. Lower socioeconomic populations in Indiana may not be as price-responsive as similar populations nationally.

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

POSTER #79

ACUTE MYELOID LEUKEMIA: DOES ONE SIZE FIT ALL? A RETROSPECTIVE ANALYSIS OF OUTCOMES OF THERAPY AT MOI TEACHING AND REFERRAL HOSPITAL IN ELDORET, KENYA

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Background

Care for acute myeloid leukemia (AML) remains challenging despite the recent advances in therapy. There remains a wide gap between the standard of care set based on clinical studies – designed and executed in high-income countries (HIC) – and the options available in low- to middle-income countries (LMIC). Institutional experience at Moi Teaching and Referral Hospital (MTRH) has consistently shown that most of the patients receiving the traditional 7+3 chemotherapy do not tolerate it well and typically die within the same hospital stay. Institutional practice has relied on low-dose cytarabine (20 mg intravenously every 12 hours for 10 days per cycle) every 4 – 6 weeks. We aimed to study the outcomes of this approach.

Methods

We analyzed data for adult patients with AML who were treated at MTRH over a period spanning 36 months between June 2012 and July 2015. Medical information was extracted from a deidentified database built for quality improvement purposes.

Results

20 patients with mean age at diagnosis 43.9 ± 17.4 years were treated for AML over the study period. 17 patients (85.0%) received low-dose cytarabine with the mean number of cycles being 1.1. One patient had received one cycle of doxorubicin and one patient had received one cycle of combination cytarabine and doxorubicin prior to low-dose cytarabine therapy. The median overall survival was 181 days (range: 1 – 721 days). 11 patients (64.7%) had completed at least one full cycle of cytarabine therapy. An analysis excluding patients lost to follow up showed a mean survival of 273.5 days for patients who completed at least one cycle of low-dose cytarabine therapy, as compared to 143.5 days for those who did not. Eighteen of the 20 patients died of the disease within one year of diagnosis. Five-year survival remains at a striking 0% as compared to rates consistently > 50% in patients < 70 years of age in HIC.

Conclusions

AML therapy results remain poor in Kenya when compared to numbers from HIC. There exists need for prospective studies in LMIC with appropriate consideration for the limited resources and the access to the most novel effective therapies after the completion of clinical trials. Improvement in hospital infrastructure to minimize the risk of infections and maximize access to blood products is also an important step to undertake in preparation for more cytotoxic and transplant therapies to be available.

Population Science/Epidemiology ***Resident***

POSTER #80

REASONS FOR ADMISSION IN PATIENTS WITH CANCER, LEUKEMIA, LYMPHOMA, AND MULTIPLE MYELOMA (RAP-CALM) AT MOI TEACHING AND REFERRAL HOSPITAL – ELDORET, KENYA: A PILOT STUDY

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Background

Patients with cancer need frequent admissions to the hospital for treatment of acute conditions and management of severe refractory symptoms. In this study, we describe the distribution of hospital admissions for patients with cancer, including malignant solid tumors, leukemia, and lymphoma, in a tertiary referral center in Eldoret, Kenya. A consultation to Hematology/Oncology is placed for patients with an active diagnosis of cancer who are admitted to Moi Teaching and Referral Hospital (MTRH).

Methods

We followed patients with an active diagnosis of cancer (solid malignant tumor, multiple myeloma, leukemia, or lymphoma) who were admitted to MTRH over the five months extending between September 2017 and January 2018. Medical information was extracted from the weekly inpatient consult service summaries. The inpatient consult summaries were updated on a weekly basis based on the primary data extracted from patients' medical records for quality improvement purposes.

Results

157 patients were admitted with a diagnosis of cancer, either established or confirmed later during the hospital stay. Table 1 shows the patient characteristics. Median length of stay was 17 days (range: 1 – 439 days). Females had a significantly longer length of stay when compared to males (38 versus 18 days, $p = 0.043$). 24.3% of patients died during the hospital stay with no statistically significant difference between the sexes. Only four patients were readmitted during the study interval. 80.5% of patients with solid tumors had stage IV disease. Most patients (13.1%) were admitted to the hospital with symptomatic anemia requiring transfusion therapy. Other reasons for admission are detailed in Table 1.

Conclusions

Despite recent advances, length of hospital stays, cancer stage at the time of presentation, and inpatient mortality rates remain high. Several factors contribute to this status quo including the current lack of radiation therapy services within the facility, the detention of patients after hospital discharge pending settlement of bills, the lack of intensive care beds, the high patient-to-nurse ratio, the lack of an electronic health record, and the need for more widespread screening and population health efforts.

Table 1. Characteristics of patients admitted to Moi Teaching and Referral Hospital in Eldoret, Kenya

Sex, % female	90 (57.3%)

Age, median in years (range)		48 years (14 – 48 years)
Cancer diagnosis, %	Breast	36 (22.9%)
	Sarcoma (angiosarcoma, rhabdomyosarcoma, synovial sarcoma, osteosarcoma)	10 (6.4%)
	Multiple myeloma	9 (5.7%)
	Acute myeloid leukemia	9 (5.7%)
	Colorectal	9 (5.7%)
	Non-Hodgkin's lymphoma	9 (5.7%)
	Chronic myelogenous leukemia	8 (5.1%)
	Hepatocellular carcinoma	7 (4.5%)
	Chronic lymphoid leukemia	6 (3.8%)
	Skin (squamous cell)	6 (3.8%)
	Gastric (adenocarcinoma)	5 (3.2%)
	Nasopharyngeal carcinoma	5 (3.2%)
	Acute lymphoblastic leukemia	4 (2.5%)
	Pancreaticobiliary (pancreatic adenocarcinoma, cholangiocarcinoma)	3 (1.9%)
	Hodgkin's lymphoma	3 (1.9%)
	Kaposi's sarcoma	3 (1.9%)
	Lung	2 (1.3%)
	Prostate	2 (1.3%)
	Malignant melanoma	2 (1.3%)
	Renal cell carcinoma	2 (1.3%)
Reasons for admission	Anemia requiring transfusion therapy	21 (13.1%)
	Respiratory failure	20 (12.5%)
	Breast infection	14 (8.8%)
	Refractory pain	10 (6.3%)
	Poor feeding, vomiting, hypovolemia	9 (5.6%)
	Bowel obstruction	8 (5%)
	Renal failure	6 (3.8%)
	Neutropenic fever	5 (3.1%)
	Severe sepsis	5 (3.1%)
	Gastrointestinal bleeding	5 (3.1%)
	Tumor lysis syndrome	2 (1.2%)
	Deep venous thrombosis	1 (0.6%)
Stage of cancer for solid malignant tumors, %	Stage I	0
	Stage II	2 (1.6%)
	Stage III	22 (17.9%)
	Stage IV	99 (80.5%)

Population Science/Epidemiology resident

POSTER #81

"HOW DID THEY NOT MENTION THAT?" -A SYSTEMATIC REVIEW OF ADOLESCENT AND YOUNG ADULT CANCER SURVIVORS' EXPERIENCES OF FERTILITY, SEXUALITY, AND ROMANTIC RELATIONSHIPS

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Problem: Adolescent and young adult (AYA) cancer survivors struggle with many psychosocial challenges that threaten their ability to achieve important developmental milestones. Some of the most distressing challenges involve infertility, poor psychosexual functioning, and strained romantic relationships. There is a knowledge deficit regarding AYA's experiences of these issues and their survivorship needs.

Purpose: The purpose of this systematic literature review was to analyze and synthesize qualitative data regarding AYA experiences of fertility, sexuality, and romantic relationships and provide recommendations for practice and research.

Search Strategy: Three databases (PubMed, PsycINFO, Ovid) were used to identify qualitative studies published between January 2007 to September 2017. Inclusion criteria: male/female cancer survivors diagnosed during adolescence or young adulthood (defined as ages 13

-29). Exclusion criteria: subjects currently on-treatment, survivors of childhood cancers, and quantitative studies.

Results of Literature Search: Fourteen studies met the inclusion criteria. We then assessed the methodological quality of each study using guidelines synthesized from two articles addressing critique of qualitative research. The results of our literature search were integrated

using tables and then evaluated with a point-based scoring system to reflect the quality of research. We then identified theme categories which illustrated the commonalities of AYAs' experiences using an abbreviated phenomenological method.

Synthesis of Evidence: After conducting a review of the literature and compiling the results, data was then analyzed. Preliminary themes were identified as (1) Poor provider communication; (2) Gender disparities; (3) Lack of control/choice; (4) Problems with disclosure & intimacy; (5) Compromised normality. The results reflected an overall lapse in current

systematic approaches to discussions of fertility in relation to cancer

treatment.

Implications for Practice: Understanding AYA cancer survivors' experiences of fertility, sexuality, and romantic relationships is important to help minimize psychosocial distress and develop strategies to help met their needs. These topics are very sensitive and minimally addressed in the current clinical setting. Preliminary recommendations point to the need for improved communication, referrals, and support for AYA survivors regarding fertility, sexuality, and quality of relationships.

Translational/Clinical Research ***BSN Student***

POSTER #82

COMMON MOLECULAR ALTERATIONS IN CANINE AND HUMAN MALIGNANT GLIOMAS: POTENTIAL NOVEL THERAPEUTIC TARGETS

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To understand and therapeutically target molecular alterations associated with human glioma, a better more representative pre-clinical animal model is required. Spontaneous canine (Canis lupus) malignant gliomas hold tremendous potential as an immunocompetent large animal model to better understand human malignant gliomas (MG). To integrate this powerful model into the pre-clinical studies it is of utmost importance to thoroughly characterize the model and understand the similarities and difference in comparison to human disease. In our recent publication, using RNAseq of canine malignant oligodendrogliomas (OG), we demonstrated that canine malignant OGs carry similar differential expression of common oncogenes that are well described in human MG. In the current study we performed comprehensive analysis of the RNAseq data to identify genes that are highly deregulated in canine malignant OG and compared their expression with human MG utilizing the publicly available database to identify novel therapeutic targets. In order to determine the protein partners interacting with altered proteins and the pathways enriched, STRING-database was utilized. RNAseq data was confirmed using qRT-PCR for the deregulated genes whose functions are known and unknown in MGs. Our results revealed that 23% genes (3,712 out of 15,895) are altered in canine malignant OG. Among these altered genes, 11% were upregulated (n=1858) and 11% were downregulated (n=1854). Top 10 significantly upregulated and downregulated genes were in the range of 12.25 to 13.72 and -9.56 to -6.46 respectively. Top 10 CD genes upregulated were in the range of 3.39 to 11.38. Selected genes from upregulated (*RRM2*, *IRX5* and *DTL*), downregulated (*SLC22A6a* and *SLC5A5*), and CD genes (*CD163*, *CD93*, *CD36* and *CD1A6*) categories from the canine tumor were confirmed by qRT-PCR. Comparison of these genes with the human database confirmed that these genes are also altered in human MG, thus indicating the molecular similarity of canine glioma with human disease. Protein interactions and pathways analysis of upregulated genes revealed the cell cycle regulatory pathway as one of the common pathway enriched in both human and canine tumor. In conclusion, we identified several common genes dysregulation in canine and human MG that can serve as potential therapeutic target and can be tested in the .preclinical canine model.

Translational/Clinical Research ***Faculty***

POSTER #83

EPIGENOMIC SIGNATURES OF ACQUIRED PLATINUM RESISTANCE IN HIGH GRADE SEROUS OVARIAN CANCER

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Epithelial ovarian cancer (OC) is the most lethal gynecologic malignancy. The majority of advanced stage patients develop uniformly fatal resistance to standard platinum-based treatments. Epigenetic changes, particularly DNA methylation aberrations have been implicated in acquired resistance to platinum in OC. The goal of the current study was to identify methylation changes associated with the development of acquired resistance to platinum-based chemotherapy. We hypothesized that OC tumors harboring an aberrant DNA methylome could be reversed by DNMTi and re-sensitized to platinum treatment. To achieve our objective, we generated and compared tumor DNA methylation profiles from patients with recurrent platinum-resistant OC treated on a clinical trial with a DNA methyl transferase inhibitor, guadecitabine, before (n=65) and after (n=19) treatment (NCT01696032), and patients with primary (platinum-naïve) OC (n=20). Human ovarian surface epithelial cells (HOSE) were used as controls (n=5). We examined methylation levels across the genome using the Infinium Human Methylation 450 Bead Chip (Illumina). By comparing the methylome of platinum naive and platinum resistant ovarian tumors, normalized to HOSE, we identified 452 CpG island-containing gene promoters that became hypermethylated in resistant tumors. Of those, 128gene promoters were hypermethylated at an FDR<0.05 and 17 genes at an FDR<0.05 and ¿ß>0.1. Among those significantly hypermethylated genes, acquired promoter CpG island methylation of *Ras Association Domain Family 1A*(*RASSF1A*; a tumor suppressor gene in multiple cancer types) was observed. Of the 451 hypermethylated promoters in tumor resistant samples, 189 CpG islands became hypomethylated after guadecitabine treatment, suggesting that these promoters may be associated with reversal of resistance. Of those were the *IFNA8* promoter, a gene known to be associated with anti-tumor effects in ovarian and other solid malignancies, and DEFB124, which is involved in the innate immune response. Pathways associated with immune reactivationwere highly enriched by guadecitabine treatment. Together, these results support that aberrant DNA methylation affecting gene networks associate with immune response plays a role in platinum resistant development and could be a therapeutic target.

Translational/Clinical Research ***Faculty***

POSTER #84

UNDERSTANDING AND EXPLOITING IB-DNQ-INDUCED DNA DAMAGE RESPONSES IN NQO1+ CANCER THERAPY

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The detoxifying enzyme NQO1 (NAD(P)H:quinone oxidoreductase 1) is a promising therapeutic target due to its overexpression in many solid cancers and very low presence in normal cells. Certain chemotherapeutic agents, such as ß-lapachone and deoxynyboquinone (DNQ), target NQO1 to induce programmed necrosis in solid tumors and have shown great promise, but they have not been able to show the full potential of an NQO1-activated anticancer agent due to their low solubility. Thus, more potent tumor-selective compounds are needed. Based on its structure and mode of action, isobutyl-deoxynyboquinone (IB-DNQ) was recently added to the spectrum of NQO1 substrates. IB-DNQ increased NQO1 processing, enhancing both the potency and the selectivity of its anticancer properties, making it a highly efficient NQO1 substrate, and thus an outstanding anticancer agent. IB-DNQ is a promising antitumor agent whose mechanism of action had previously not been elucidated. Here, we show that IB-DNQ kills cancer cells in an NQO1-dependent manner with even greater potency than ß-lapachone and DNQ. IB-DNQ treatment caused extensive DNA damage, PARP1 hyperactivation, and severe NAD^+/ATP depletion leading to μ-calpain-mediated cell death. Significant antitumor efficacy and prolonged survival were noted in human orthotopic pancreatic and non-small cell lung cancer (NSCLC) xenograft models. PARP1 hyperactivation and dramatic ATP loss were noted in the tumor, but not in the associated normal lung tissue. These findings offer a preclinical proof-of-concept for IB-DNQ as a potent chemotherapeutic agent for the treatment of various $NQO1^+$ cancers.

Translational/Clinical Research ***Faculty***

POSTER #85

EPIGENETIC REPROGRAMMING OF H3K27ME3 VIA EZH2 INHIBITION POTENTIATES STANDARD-OF-CARE-MEDIATED GROWTH INHIBITION IN EWING'S SARCOMA

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Therapies for relapsed Ewing's Sarcoma Family of Tumors (ESFT) have not improved in the past 25 years. Current standard-of-care (SOC) agents result in 70% survival in patients with localized ESFTs, but only 15-20% in relapsed patients. Approximately 85% of ESFTs have the chromosomal translocation t(11; 22) (q24;q12) which encodes for the oncogenic EWS/FLI1 transcription factor leading to aberrant regulation of genes that promote tumorigenesis and therapeutic resistance. A key downstream target of EWS/FLI1 is the enhancer of Zeste Homolog 2 (EZH2), an epigenetic regulator that is the catalytic component of the polycomb repressor complex 2 (PRC2). EZH2 is overexpressed in ESFT and maintains tumor oncogenicity by tri-methylating histone 3 lysine 27 (H3K27me3) to modulate gene expression. Genomic sequencing data from the Pediatric Cancer Precision Medicine Program at the Indiana University School of Medicine in partnership with Riley Precision Genomics showed increased EZH2 RNA in 4 of 5 EWS/FLI1^{+} ESFT clinical biopsies. Others reported that elevated EZH2 in ESFTs and other cancers correlated with increased therapeutic resistance. We *hypothesize* that EZH2 contributes to therapeutic resistance in ESFTs by dysregulation of critical survival genes (p21 and c-MYC), and pharmacological inhibition of EZH2 will enhance sensitivity to the cytotoxic effects of SOC. Increased EWS/FLI1 and EZH2 expression was observed in molecularly characterized pediatric ESFT cell lines and xenografts compared to controls (p<0.001), and correlated with elevated H3K27me3 (p<0.001). *In vitro* EZH2 inhibition via tazemetostat showed a significant reduction of H3K27me3 by one day post-treatment which was sustained or completely reduced by 7-days post-treatment (p<0.001). In ESFT xenografts, tazemetostat decreased H3K27me3 by 5 and 10-days post-treatment (vehicle vs treated, p<0.001). Decreased tumor growth was evident at day-5 post-treatment with tazemetostat, but tumor growth resumed by day-10. *In-vitro* cell growth assays indicated tazemetostat potentiated etoposide-mediated growth inhibition versus single agents alone(p<0.001). Inhibition of EZH2 resulted in up-regulation of p21 (p<0.05) and down-regulation of c-MYC (p<0.01) transcripts in all ESFT cell lines. Systematic investigation of EZH2 inhibition in combination with SOC provides rationale to pursue combination therapy in EZH2-expressing ESFTs to overcome therapeutic resistance.

Translational/Clinical Research ***Faculty***

POSTER #86

THE ANTI-ESTROGEN ENDOXIFEN ALTERED BONE MORPHOLOGY AND REDUCED MUSCLE FUNCTION IN MICE

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Endoxifen, the predominant CYP2D6 metabolite of the selective estrogen receptor modulator (SERM) tamoxifen, is currently being developed as a novel anti-estrogen therapy for the treatment of estrogen receptor (ER)+ breast cancer. Genetic polymorphism of CYP2D6 is a predictor of response to tamoxifen in patients, implicating endoxifen as one of the most active and relevant tamoxifen metabolites. Breast cancer patients treated with adjuvant endocrine therapies including aromatase inhibitors (AIs) and SERMs often report unmanageable musculoskeletal toxicities that can result in treatment discontinuation. While ER binding affinity and anti-tumor effects have been established in preclinical models, the effects of endoxifen on the musculoskeletal system are not fully known. Twenty-week female C57BL/6 mice underwent sham surgery or ovariectomy (OVX) and were treated daily with vehicle, the AI letrozole, or the SERM endoxifen. Body composition was assessed prospectively by DXA. Bone indices and marrow adipose tissue volume were measured by μCT and muscle contractility of the extensor digitorum longus (EDL) was measured *ex vivo*. After eight weeks, trabecular bone volume fraction (BV/TV) decreased by 50% in OVX-vehicle and OVX-letrozole mice, whereas BV/TV increased threefold in endoxifen mice relative to sham-vehicle. Despite the presence of significantly more trabecular bone following endoxifen treatment, cortical bone analyses revealed impaired periosteal and endosteal expansion of bone, resulting in reduced polar moment of inertia, which is a measure of resistance to fracture. Uterine weight increased significantly in endoxifen-treated mice relative to all OVX groups, similar to what is observed with tamoxifen treatment. Peripheral body fat, bone marrow adipose tissue, and circulating leptin were reduced by endoxifen, suggesting that the drug may elicit positive systemic metabolic effects. At the termination of the study, muscle-specific force was reduced in OVX-endoxifen mice relative to sham-vehicle, OVX-vehicle, and OVX-AI mice, despite no change in muscle mass. While endoxifen shows promise as a potent anti-estrogen therapy for the treatment of ER+ breast cancer, it may be important to monitor patients for endometrial proliferation, morphological changes in bone that increase fracture risk, and for musculoskeletal side effects that could reduce drug compliance.

Translational/Clinical Research ***Faculty***

POSTER #87

INTRAOPERATIVE ASSESSMENT OF TUMOR MARGINS DURING GLIOMA RESECTION BY DESI-MS

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Microsurgical resection aims to maximize tumor excision, with evidence suggesting a correlation between maximal resection and increased survival rates. Consequently, accurate assessment of the negative margin of gliomas is of paramount importance. That said, gliomas exhibit fibrous, often difficult to discern, borders that render complete resection challenging. Surgical margins, often defined on the basis of surgical experience, visual observation, and neuronavigation, are rarely determined intraoperatively. While histology remains a diagnostic gold standard from which further molecular testing and clinical decisions follow, it is not performed concurrently with tumor resection due to its time-intensive nature, rendering current protocols unable to elucidate surgical margins in “real-time”. Consequently, we propose the development of a method to evaluate surgical margins rapidly on the basis of molecular information using desorption electrospray ionization-mass spectrometry (DESI-MS), and integrate this information with standard of care imaging techniques like MRI that determine surgical margins on the basis of differential contrast-enhancement between the tumor mass and adjacent tissue. DESI-MS is a morphologically-friendly, atmospheric pressure ionization method that requires no sample preparation and/or separation and is capable of point-of-care testing, performing measurements in the operating room within three minutes. Biopsied tissue specimens from surgeon-defined positions in the tumor and walls of the resection cavity are smeared onto glass microscope slides and sprayed with charged solvent droplets to extract molecules from the tissue surface. Intraoperative DESI-MS is used to characterize tissue smears by comparison with a library of DESI mass spectra of pathologically determined tissue types. Determination of tumor infiltration into white or grey brain matter is based on measurements of *N*-acetylaspartate (NAA) and membrane-derived complex lipids, with results subsequently confirmed by histopathology using the same tissue smear analyzed by DESI-MS. It is observed that measured levels of NAA signal intensity decrease exponentially as pathologically measured tumor cell percentage (TCP), a measure of tumor infiltration, increases. Results for 253 biopsies from 38 subjects who underwent glioma resection show that DESI-MS allows for the detection of glioma and estimation of high TCP on the basis of NAA and lipids with an average sensitivity of 80% and an average specificity of 77%, with efforts being made to improve these values. This work demonstrates the strength of DESI-MS in assessing surgical margins for maximizing safe and effective tumor resection, with further validation after the performance of a clinical trial. With the information provided by this technique not available with standard procedures and evidence to suggest that tumor infiltration extends beyond the MRI contrast images, it is possible that this method can complement this aspecific information with direct examination of the resected tissue. While DESI-MS could be easily inserted in the conventional surgical workflow with no alterations, protocol standardization and automation will further ease this implementation.

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

POSTER #88

JAK2 REGULATES OXIDATIVE DAMAGE-INDUCED EPIGENETIC ALTERATIONS

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Background: At sites of chronic inflammation, surrounding epithelial cells undergo aberrant DNA methylation, contributing to tumorigenesis. Inflammation is associated with an increase in reactive oxygen species (ROS) that cause oxidative DNA damage which has also been linked to epigenetic alterations. We previously demonstrated that in response to ROS exposure, mismatch repair proteins (MMR) MSH2 and MSH6 recruit epigenetic silencing proteins DNA methyltransferase 1 (DNMT1) and Polycomb repressive complex 2 (PRC2) members to sites of DNA damage, resulting in transcriptional repression of tumor suppressor genes (TSGs). In addition to inducing DNA damage, ROS activates several kinases that may participate in the DNA repair and epigenetic responses. We hypothesize that in response to ROS, JAK2 is rapidly activated and translocates to the nucleus where it mediates recruitment of mismatch repair (MMR) proteins and epigenetic silencing proteins to damaged chromatin, and thereby regulates repressive epigenetic alterations and gene transcription in response to oxidative damage.

Method: Nuclear extraction and immunofluorescence were used to determine alterations in subcellular localization of JAK2 in response to hydrogen peroxide (H_2O_2). To detect changes in protein-protein interactions or chromatin binding following H_2O_2 in combination with JAK2 inhibition or knockdown, we used immunofluorescence, co-immunoprecipitation (Co-IP) and chromatin affinity assays. Whole cell lysate and qRT-PCR were used to examine downstream histone modifications and transcription changes, respectively.

Results: We uniquely demonstrate that in response to H_2O_2, JAK2 localizes to the nucleus and interacts with MSH2 and MSH6. Inhibition or knockdown of JAK2 reduces the H_2O_2-induced chromatin interaction of MSH2, MSH6, DNMT1 and PRC2 members, as well as H_2O_2-induced global increase in trimethylation of lysine 27 of histone H3 (H3K27me3). JAK2 also regulates oxidative damage-induced transcriptional repression of candidate TSGs. Moreover, JAK2 mRNA expression is associated with CpG island methylator phenotype (CIMP) status in colorectal cancer.

Conclusions: These findings suggest active JAK2 translocates into the nucleus in response to oxidative damage and nuclear JAK2 plays a key role in initiating oxidative damage-induced epigenetic alterations. Our findings provide novel insight into the connection between kinase activation and epigenetic alterations during oxidative damage and inflammation.

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

POSTER #89

PIK3CA E542K MUTATION IN BLADDER CANCER CONFERS RESISTANCE TO PI3K TARGETED THERAPY BUT SYNERGY WITH BET INHIBITION

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Background: We have previously reported that two bladder cancer PDX models that carry distinct *PIK3CA* helical domain (HD) mutations respond differently to dual PI3K/mTOR inhibitor (LY3023414), where one model is sensitive (RP-B-02, PIK3CA *E545K MUT*) while the other is resistant (*PIK3CA*, *E542K MUT*) (Wei L *et al* Oncotarget 2016). Thus, we transduced these mutations in isogeneic cells to determine whether each mutation is indeed associated with a distinct oncogenic potential, as well as differential therapeutic response. Subsequently, we tested rational drug combinations capable of overriding resistance to PI3K targeted therapy (i.e. bromodomain inhibitor). **Methods**: Isogeneic HEK cells and urothelial cells (SV-HUC) were transduced with plasmids carrying either wild type (WT) or mutant *PIK3CA* (E545K and E542K mutants). Proliferation and growth were compared both in 2D and 3D culture respectively. We tested the therapeutic response to LY3023414 in 2D and 3D culture. Western blotting was used to test target modulation in the PI3K/mTOR pathway in response to treatment. JQ1 and LY3023414 combination studies were done *in vitro.* Calcusyn™ was used to determine whether synergy between the two agents exists in the context of either mutation. **Results:** *PIK3CA* E542K mutation has a growth advantage compared to the E545K mutation. This observation was more significant in 3D culture, which more faithfully represents the complexity of the tumor microenvironment. Isogeneic HEK cells expressing the E542K mutation were significantly more resistant to LY3023414 treatment both in 3D and 2D. This resonates with the resistance we initially reported in the bladder cancer PDX models carrying the E542K mutation. Interestingly, LY3023414+JQ1 combination was synergistic in the RP-B-01 (E542K *MUT,*but not in the RP-B-02 cell line (E545K *MUT*). **Conclusion:** *PIK3CA* mutation status is predictive of response to PI3K targeted therapy in bladder cancer, where *PIK3CA* E542K mutation confers resistance to PI3K targeted therapy. The synergistic combination of LY3023414 and the bromodomain inhibitor JQ1 can override resistance to PI3K targeted therapy. This drug combination may be of clinical significance in the context of *PIK3CA* E542K, but not E545K mutation.

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

POSTER #90

CAF-INDUCED OVARIAN CANCER STEM CELLS IMPART CHEMORESISTANCE IN OVARIAN CANCER

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Ovarian cancer is the most lethal gynecologic cancer and 5th leading cause for cancer related deaths among women in America in 2016. Most patients develop resistance to conventional carbo-taxol based treatment and eventually succumb to chemoresistant disease. Cancer stem cells are resistant to cytotoxic chemotherapy, therefore, they survive and causes relapse. The tumor microenvironment provides relevant factors essential for cancer stem cell maintenance. Cancer associated fibroblasts (CAFs) are one of the main constituents of the tumor microenvironment in ovarian tumors, forming 10-50% of the tumor mass. There is increasing evidence that CAFs promote tumor progression and chemoresistance. Here we focus on the role of CAFs in maintaining/promoting ovarian cancer stem cell population and its effect on chemoresistance.

Co-culture of ovarian cancer cells with CAFs resulted in increased resistance to carboplatin, which could potentially be due to an induction of cancer stem cells by the CAFs. Mimicking the tumor microenvironment, we co-cultured ovarian cancer cells with CAFs and found an increase in ALDH positive ovarian cancer cell population, which is a reliable marker for ovarian cancer stem cells. Furthermore, we co-cultured ALDH negative ovarian cancer cells with CAFs and observed that CAFs have the ability to convert a subpopulation of non-cancer stem cells into cancer stem cells. Ovarian cancer cells co-cultured with CAFs showed elevated ALDH expression as well as spheroid formation ability. Conditioned medium from CAFs did not alter ALDH expression, which indicates that cancer stem cell induction by CAF is dependent on juxtacrine interactions or close contact. Our study will identify the mechanisms by which CAFs increase stemness in ovarian cancer cells and will target the relevant pathway as a novel therapeutic approach to treat chemoresistant disease.

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

POSTER #91

RETROSPECTIVE OUTCOMES OF PROLONGED-DURATION CALCINEURIN INHIBITOR IMMUNOSUPPRESSION IN SEVERE APLASTIC ANEMIA

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Background
Most research protocols specify a 6-month course of cyclosporine for severe aplastic anemia (SAA) patients treated with immunosuppression therapy, and it has become a general clinical consensus to treat for at least this long. However, there is no clear evidence-based consensus of the optimal duration or tapering regimen of calcineurin inhibition.

Hypothesis
(1) Peripheral blood counts and survival of patients with SAA treated with extended calcineurin inhibition (ECI) therapy may be comparable to those treated with matched related-donor bone marrow transplantation (MRD-BMT). (2) Patients in the ECI cohort may achieve higher peripheral blood counts than their 6-month values.

Methods
Patients were selected from a database of fifty-three patients with severe and very severe aplastic anemia treated between 1994 and 2017 at the Indiana University Melvin and Bren Simon Cancer Center. Treatment groups included ECI (defined as treatment greater than six months; n=15) and MRD-BMT (n=9).

Patients in the ECI arm were initially treated with anti-thymocyte globulin, glucocorticoids, and a calcineurin inhibitor (cyclosporine or tacrolimus) followed by continuous calcineurin inhibition for longer than 6 months. White blood cell (WBC), hemoglobin (Hb), and platelet (Plt) counts were monitored retrospectively and improvement from the pre-treatment values was analyzed using the Mann-Whitney nonparametric U test.

Results
Median follow-up time was 34mo and 54mo respectively for the ECI and BMT cohorts. Patients with severe aplastic anemia who received MRD-BMT had a statistically significant greater improvement in median WBC counts from their pre-treatment values for up to 15 months, hemoglobin for up to 7 months, and platelet counts for up to 48 months compared to those who received ECI. However, beyond these times the difference in outcomes was not significant. Survival for ECI group was 88% versus 89% for patients undergoing BMT, p-value 0.749. Patients with severe aplastic anemia who were treated with ECI had a statistically significant improvement in their hemoglobin beginning at 30 months and an otherwise general non-significant improvement in their counts.

Conclusions
In this population of patients with severe aplastic anemia, MRD-BMT demonstrated greater improvement of peripheral counts compared to ECI initially, but the difference eventually became not significant. Patients who received ECI demonstrated a consistent but non-significant improvement in their counts, except for perhaps hemoglobin, when compared to their 6 month values. The potentially comparable long-term outcomes in cell counts we have shown between ECI and MRD-BMT will be particularly relevant to patients who do not have a matched sibling bone marrow donor, have relative contraindications to a second course of intensive immunosuppressive therapy, or are not able to access a promising investigational protocol.

Translational/Clinical Research *Graduate Student*

POSTER #92

ENGINEERING PEPTIDE TARGETING LIPOSOMAL DRUG DELIVERY TO IMPROVE SELECTIVITY FOR HER2-OVEREXPRESSING BREAST CANCER

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Breast cancer is the second leading cancer in the U.S. women for whom approximately 15% of them have the likelihood of risk in development of breast cancer in their life-time. Breast cancer has largely been classified into four subtypes based upon the expression of receptor molecules closely related with cancer cell growth, progression, and survival. Among the receptors, human epidermal growth factor receptor 2 (HER2) is the most widely known biomarker in the breast cancer development and has attracted scientist's interest as a therapeutic target due to its overexpression in approximately 30% of the breast cancer patients. Trastuzumab is the FDA approved anti-HER2 humanized monoclonal antibody that has been used as the first-line treatment in HER2-positive breast cancer. Despite the successful outcomes of its use in HER2-positive breast cancer, Trastuzumab possibly gives rise to non-selectivity issues such as off-target effects owing to omnipresence of HER2 in most epithelial cells and its high binding affinity (~low nM). In this study, we utilized HER2 specific and moderate affinity peptide (named AHNP peptide, ~low μM) presenting liposome to not only achieve enhancement in HER2-overexpressing cancer cellular uptake but also reduce off-target effects by controlling surface peptide density, peptide linker length, and the size of liposome. Our results from *in vitro* cellular uptake studies showed significant uptake enhancement in HER2-overexperessing (SK-BR-3 and BT-474) over HER2-negative human breast cancer cell line (MCF 7) at 1% AHNP peptide density on 50 nm liposome and 0.5% AHNP peptide density on 100 nm liposome. In addition, we obtained significant liposome uptake enhancement in mouse tumor mammary epithelial cells at lower linker (EG_8) length of peptide in a similar manner of uptake in HER2-overexpressing human breast cancer cell lines. We also utilized allograft transplantation mouse model to investigate whether cellular uptake at breast tumor site is enhanced by AHNP targeting ligand presenting liposome while off-target effect is minimized. The results from *in vivo* study showed ~2-fold improvement in breast cancer cellular uptake at 0.3% AHNP presenting 50 nm liposome while the accumulation at tumor site is not statistically different from non-targeted liposome. The overall results suggest that AHNP targeting liposome could be potentially used for therapeutic drug delivery system in that it could selectively targets breast cancer cells by the accurately controlled peptide density. The further studies will include optimization of the AHNP liposomal drug delivery system by the modification of peptide linker, the number of lysines, and the size of liposome.

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

POSTER #93

TARGETING THE APE/REF-1 NODAL AXIS IN BLADDER CANCER

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Introduction:

Bladder cancer is the 9th most common cancer in the world with 380,000 new cases a year and 15,000 deaths. Urothelial carcinoma represents more than 90% of cases with 75-85% of tumors presenting as Non-Muscle Invasive bladder cancer (NMIBC) at time of diagnosis. Approximately 60-70% of NMIBC recurs within one year and 10-20% of cases will progress to Muscle Invasive Bladder Cancer (MIBC). With a much worse prognosis, MIBC has a survival rate of less than 50% after 5 years and has limited therapeutic options compared to the non-muscle invasive form. Cisplatin-based chemotherapy is the standard of care but despite having great initial success, many patients develop resistance to the drug. Therefore, there is a need to explore new therapeutic targets that may sensitize bladder cancer to cisplatin.

Apurinic/apyrimidinic Endonuclease/Redox Factor-1 (APE1/Ref-1) plays a key role in the base excision repair (BER) pathway and acts as a redox signaling protein that directly modulates the activity of certain transcription factors including Signal Transducer and Activator of Transcription 3 (STAT3). STAT3 is activated in a number of cancers and has been associated with cancer progression and metastasis. We propose that APE1/Ref-1activates STAT3 and drives cancer cell proliferation and survival in cisplatin-resistant bladder cancer.

Methods:

Cells were cultured in RPMI media (10% FBS). Established bladder cancer cell lines T24, UC3 and SCaBER and patient-derived bladder cell lines BLCAb001 and BLCAb002 were treated with increasing concentrations of redox-specific inhibitors APX2014 or APX2009 for 72 hours (N=4) with cell number being determined via methylene blue. BLCAb001 and BLCAb002 were treated with APX2014 and cisplatin for 72 hours with cell number being determined via alamar blue. APE1/Ref-1 was knocked down in T24, UC3, BLCAb001 and BLCAb002 cell lines using 50 nM siRNA. Knockdown was verified via western blottingand xCELLigence was used for real-time analysis of cell growth over 4 days.

Results:

APX2009 and APX2014 caused a concentration-dependent decrease in cell number in T24, UC3, SCaBER, BLCAb001 and BLCAb002 cell lines (p-value£0.05). Combination assays in BLCAb001 and BLCAb002s with APX2014 and cisplatin showed synergistic activity at a Combination Index < 1.0. Knockdown of APE1/Ref-1 was found to be >80% and bladder cancer cell growth was significantly decreased compared to scrambled control (p-value£0.05).

Conclusion:

Cisplatin-resistance is relatively common in MIBC and proves challenging to treat. APE1/Ref-1 is a multifunctional protein with both DNA repair and redox regulation activity. Inhibition of APE1/Ref-1's redox function with either specific inhibitors or siRNA decreased bladder cancer cell growth and sensitivity to cisplatin. This supports APE1/Ref-1 as a viable drug target in MIBC.

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

POSTER #94

GENETIC DISRUPTION OF RAC1 REDUCES TUMOR SIZE AND NUMBER IN A MOUSE MODEL OF NEUROFIBROMATOSIS TYPE 1

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Neurofibromatosis Type 1 (NF1) is a highly penetrant autosomal dominant genetic disorder affecting 1:3000 individuals where mutations in the tumor suppressor gene *NF1* leads to decreased neurofibromin. The most debilitating morbidity associated with NF1 is the presence of complex multiple lineage plexiform neurofibromas (pNF), slow growing Schwann cell derived tumors that can cause serious comorbidities. In addition to Schwann cells, bone marrow derived mast cells are the main driver of tumorigenesis in NF1. Decreases in neurofibromin leads to constitutively active Ras, thereby promoting survival, proliferation, and invasion. Unfortunately, there is little clinical success as these tumors are unable to be resected due to location, and only mild success with standard chemotherapeutic treatments. Our studies are focused on identifying new therapeutic targets for the treatment of pNF.

Our hypothesis is that Rho family GTPases, including Rac1, a key node of the Ras pathway that modulates both Ras-Raf-Mek-Erk signals as well as Ras-PI3K pathway signals, are viable targets to disrupt pNF formation and progression. Increases in GTP-bound Rac1 leads to deregulation and increased cell motility and proliferation, a hallmark for cancer cell invasion and metastasis.

We developed a novel genetically engineered mouse model (GEMM) of NF1 wherein a neural crest specific *Postn*cre targeted the floxed *Nf1*overcoming the embryonic lethality of full loss of *Nf1* and recapitulating the pNF found in patients. In this study, we determined that the additional loss of *Rac1* in the Schwann cells was sufficient to prevent tumor formation ($Nf1^{f/f}$ *Postn*Cre^{+} vs $Nf1^{f/f}Rac1^{f/f}$*Postn*Cre^{+}). Furthermore, loss of *Rac1* in Schwann cells decreased the number of infiltrating mast cells found within the proximal nerve.

Decreased mast cells within the proximal nerve lead to a second GEMM developed to further elucidate the role of*Rac1* in the pNF microenvironment. *Krox20*mice were irradiated and transplanted with either $Nf1^{+/-}$ $Rac1^{+/+}$*Mx* cre or $Nf1^{+/-}Rac1^{f/f}$*Mx* cre bone marrow following 4 injections of polyinosinic:polycytidylic acid (pIpC) to activate the cre. Disruption of *Rac1*in the bone marrow significantly reduced the tumor number per mouse. Additionally, in vitro studies showed that the secondary loss of *Rac1* in the bone marrow derived mast cells restored colony formation and migration to WT levels.

We have shown that the additional loss of *Rac1* in either the pNF cell of origin (Schwann cell) or the known driver of pNF progression (mast cells) reduced or prevented pNF formation. Our next step is to find compounds that target *Rac1*and study them in vivo. These studies provide crucial characterization of the development and progression of pNF that can be further used to develop novel compounds for the treatment of NF1 and other Rasopathies.

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

POSTER #95

CANINE CAR T-CELLS THERAPY FOR MAMMARY CARCINOMA IN DOGS

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Canine CAR T-cells therapy for mammary carcinoma in dogs

Xavier E. Ramos-Cardona, Yong Lee, Phillip Low, and Sulma Mohammed

In women, ductal carcinoma in situ (DCIS) is an often-diagnosed breast disease that is widely considered to be a non-obligate precursor of invasive carcinoma. However, the rareness of triple-negative DCIS (TN-DCIS) in women suggests that triple-negative breast cancer (TNBC) may not occur through the DCIS pathway or may simply evolve through a very short and relatively undetectable non-invasive stage. TNBC is the most aggressive and lethal form of breast cancer and predominantly occurring in women at high genetic risk including those with mutated BRCA1 genes. The rareness of detecting human TN-DCIS has hindered a better understanding of the early molecular events involved in the evolution and invasion of TNBC and has also limited the prospect of preventing this lethal breast cancer variant. We provide for the first time an immunocompetent animal model for TN-DCISthat will facilitate molecular analysis of pre-invasive TNBC and will provide an invaluable resource for identifying and selecting targets for TNBC vaccine immunoprevention or immunotherapeutic intervention.

Unlike most studied rodent models, dogs develop TN-DCIS spontaneously without genetic or chemical manipulation. We have shown that canine DCIS resembles human DCIS with shared histopathologic and molecular features and with similar imaging and behavioral characteristics. Dogs are much more outbred than laboratory rodents, yet certain breeds are at increased risk for developing mammary tumors. Indeed, we have found that 50% of randomly screened asymptomatic hound dogs have premalignant mammary lesions and that mammary TN-DCIS often progresses to invasive carcinoma within one year. Given the many common features of canine breast cancer and the high homology between the canine and human genome, the dog model offers an outstanding opportunity for exploiting TNBC immunoprevention and immunotherapeutic strategies. Moreover, the prevalence and rapid progression of canine TN-DCIS provides a much more rapid and cost-effective alternative to human trials for evaluation of the clinical effectiveness of cancer vaccine strategies.

One immunotherapeutic strategy we are testing using our canine TN-DCIS model is chimeric antigen receptor (CAR) T-cells. CAR T-cells have shown promise in treating many malignancies, but in solid tumors has been hindered by many limitations. To overcome these limitations, in collaboration with Dr. Low, we designed a genetically engineered universal canine CAR T-cells that must be activated and targeted by a small molecule adaptor before it can kill cancer cells. Our results showed that universal CAR T cells is functional and killed canine mammary tumor cell lines *in vitro*.

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

POSTER #96

FOLATE TARGETED INTRAOPERATIVE FLUORESCENCE IN LAPAROSCOPIC PARTIAL NEPHRECTOMY

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Background

Evaluating the difference in Folate Receptor a (FRa) expression in both normal kidney tissue and renal cell tumors may help with improving negative margins and detection of residual tumor both during and immediately following excision. Coupling the folate analog with OTL38 with the fluorescent tracer indocyanine green can help identify the local extent of the tumor before excision due to the difference in folate receptor expression in parenchyma versus renal tumors. Since FRa is highly expressed in normal kidneys and less so in renal tumors, fluorescence in the parenchyma and high intensity staining of the renal tubules is expected; correspondingly, renal tumor will not fluoresce and will have low intensity staining.

Methods

In this nonrandomized phase 2 clinical trial, 10 patients, each with localized renal cell carcinoma (RCC), undergoing robot-assisted laparoscopic partial nephrectomy (RALPN) had tumor and surrounding normal kidney tissue collected. These tissues were evaluated to quantify the difference in FRa expression in the parenchyma and tumor as well as evaluate the staining intensity between the normal renal tubules and the tumor cells.

Results

The patients demonstrated localized staining of the proximal renal tubules, confirming higher FRa expression in the parenchyma than in the tumor. Using a paired t-test to compare the staining in the tumor versus the parenchyma for each of the 10 patients, a statistically significant difference was found between the staining of the tumor (M=0.04669, SD= 0.0318) and the normal tissue (M=0.2086, SD= 0.1030); p=0.001602. Correspondingly, there is a statistically significant difference in the FRa expression between the RCC tumor and the kidney parenchyma.

Conclusion

The immunohistochemistry results confirmed the fluorescence seen during surgery, proving a statistically significant difference between the higher FRa expression and staining in normal kidney tissue than in the RCC tumor. These results support the use of folate analogs coupled with fluorescent tracers as a viable means to improve excision margins during RALPN surgery.

Translational/Clinical Research *Graduate Student*

POSTER #97

SELECTIVE TARGETING OF ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) VIA CD22 TARGETING LIPOSOMAL NANOPARTICLES

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Leukemia is a type of blood cancer caused by the increased number of abnormal white blood cells that is detrimental to cause death for many patients. The severity of leukemia has been rising, and the mortality rate in the patients is significant today. Regarding this severity, several first-line treatments have been developed, such as imatinib mesylate. However, these treatments induce numerous side effects, such as severe deficiency in blood cell counts because not only they target leukemia cells but also healthy cells as well. Therefore, it is critical to develop therapeutics that only target leukemia cells to reduce systematic toxicity in patients. Here, we demonstrate rationally engineered multifunctional liposome model developed from our lab that presents leukemia cell specific targeting ligands on its surface to selectively target leukemia cells over healthy cells. Among the many types of leukemia, we specifically focused on acute lymphoblastic leukemia (ALL) which is the most common type of leukemia among the children in the United States. Throughout numerous studies in the past, CD22 has been validated as a potential target for B-cell ALL and after analyzing the 3D crystal structure (PDB ID : 5VL3) of the interaction between CD22 and its therapeutic antibody, Epratuzumab, we isolated potential CD22 binding peptide sequence. Utilizing this short peptide and presenting on liposome platform, we performed cellular uptake assays on two different cell lines, Raji (CD22+) and Jurkat (CD22-). The results showed significant uptake in Raji starting at 4% peptide density comparing to Jurkat. Furthermore, the peptide was determined to be specific as the result of competitive-binding assay. In spite of the selectivity on CD22, there still need numerous improvements and modifications on our model to enhance further selectivity. Regarding this purpose, we are going to investigate another receptors that could be potentially dual-targeted with CD22 to achieve synergistic effects to further selectively target leukemia cells. Altogether, this model will potentially enable the disease driven engineering of a nanoparticle-based drug delivery system in the treatment of leukemia.

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

POSTER #98

UNDERSTANDING AND EXPLOITING IB-DNQ-INDUCED DNA DAMAGE RESPONSES IN NQO1+ CANCER THERAPY.

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NAD(P)H:quinone oxidoreductase (NQO1) abrogates toxic quinone ingestion via 2-electron oxidoreduction followed by conjugation to glutathione or glucuronic acid, which mediates excretion from cells. NQO1 also mediates activation of a select number of bioactivatable drugs to cause cytotoxic damage and cancer cell death.[1] NQO1 is overexpressed in many types of solid cancers (e.g., nonsmall cell lung, pancreatic and breast cancers) due to oncogenic and cellular stress. A novel anti-cancer bioactivatable agent, Isobutyldeoxynyboquinone (IB-DNQ) was recently developed by Hergenrother (UI-Urbana Champaign) and Boothman laboratories to be a specific substrate for NAD(P)H:quinone oxidoreductase (NQO1), roughly 7-fold more potent affinity that ß-lapachone (ARQ761 in clinical form). Bioactivatable drugs, IB-DNQ and ß-lapachone, work by causing futile redox cycling which results in DNA damage from ROS (excessive H_2O_2 production) that causes PARP hyperactivation and leads to NAD^+ depletion and subsequent programmed necrosis (NAD^+-keresis) of $NQO1^+$ cancer cells. DNA damage resulting from use of these drugs leads to excessive activation (hyperactivation) of PARP1, resulting in massive NAD^+ and ATP losses and caspase-independent (i.e., calpain-mediated) cell death.[2] A recent and novel treatment combining ß-lapachone with FDA approved PARP inhibitor (Rucaparib) showed significant synergy (***Huang et al, 2017)***. Inhibition of PARP by Rucaparib followed immediately by ß-lapachone addition prevented repair of ssDNA breaks caused by ROS, resulting in dsDNA breaks. Cell death switched from tumor death caused by programmed necrosis to tumor-mediated apoptosis.[3] Here, we show that IB-DNQ is 7-fold more potent than ß-lapachone, as well as being effective against triple-negative breast cancer (TNBC), Pancreatic Ductal AdenoCarcinoma (PDAC) and nonsmall cell lung cancer (NSCLC) cancer cells. The most efficacious, sublethal dose, as well as the minimum exposure time to death are also detailed. Effects on DNA damage and repair are shown in addition to *in vivo* efficacy of the drug against NSCLC and PDAC xenografts. Finally, we show that IB-DNQ and PARP inhibition combination therapy is more effective than IB-DNQ treatment alone.*This work was funded by NIH/NCI R01 CA221158-01 to DAB.*

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

POSTER #99

ß-LAPACHONE AS A TARGETED THERAPY THAT SELECTIVELY KILLS HIGH NAD(P)H:QUINONE OXIDOREDUCTASE 1 EXPRESSION ATYPICAL TERATOID RHABDOID TUMORS

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Pediatric brain cancers, including atypical teratoid rhabdoid tumors (ATRTs), gliomas and medulloblastomas are neoplasms of the central nervous system, and a leading cause of death in children. Although rare, ATRTs are fast growing and the symptoms progress rapidly. Approximately 20% of all the cancers in children up to 14 years old are ATRTs, as the second common solid tumors in children. Most ATRT patients receive surgery, chemotherapy and radiation; however, the survival rate is still poor. New therapies are needed to improve the survival rate and to cure the children who cannot receive surgery when they have the disease. ß-Lapachone, an NAD(P)H:quinone oxidoreductase 1 (NQO1) bioactivatable drug, as the targeted therapy selectively kills solid ATRT cancers that over-express NQO1. We demonstrate that >70% of ATRA cancers over-express NQO1. The potent antitumor activity has been shown in other cancers, such as non-small-cell lung, pancreatic, prostate and breast cancers, but remains unclear in ATRTs. NQO1 is highly expressed in ATRT cell lines and pediatric brain tissue, while NQO1 is not expressed in normal brain tissue. ß-Lapachone is efficacious against ATRTs that overexpressed NQO1, where more than 10 to 100-fold increases in NQO1 activities above low NQO1 expression in normal tissues were noted. ß-Lapachone triggers Redox cycling in an NQO1 manner, causes hyperactivation of PARP1 and programmed necrosis of ATRT cancer cells. In addition, the survival rate was improved in ATRT xenograft models using iv injected ß-lapachone-HPßCD (5X injections, eod). Together with its selectively antitumor effects, ß-lapachone may be a promising lead targeted therapy for ATRTs. ***This work was supported by an Alex Lemonade Support Grant.***

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

POSTER #100

BODY COMPOSITION CHANGES AND CACHEXIA PHENOTYPES IN PATIENTS WITH PANCREATIC ADENOCARCINOMA ON GEMCITABINE/NAB-PACLITAXEL CHEMOTHERAPY

Andrew Young[1], Safi Shahda[1,3,4], Kristen Link[1], Leonidas Koniaris[2,3,4], Teresa Zimmers[2,3,5,4]

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Background: Patients with pancreatic ductal adenocarcinoma (PDAC) exhibit progressive weight loss due to wasting of adipose tissue and skeletal muscle, known as cachexia. Cachexia reduces treatment response, increases treatment toxicity, and reduces survival. Indeed, cachexia is thought to cause death in a third of cancer patients. Cachexia is initiated by the tumor but can be exacerbated by particular chemotherapy regimens. Previously we reported that patients with PDAC on FOLFIRINOX chemotherapy exhibited three phenotypes—no wasting, fat-only wasting, or fat-and-muscle wasting. Wasting correlated with mortality, but not with tumor response to therapy. Here we queried changes in body composition in patients on gemcitabine/nab-paclitaxel chemotherapy, an alternative first-line treatment for PDAC.

Methods: This was a retrospective study of up to 141 patients with advanced PDAC treated with first-line gemcitabine/nab-paclitaxel at Indiana University. Demographic, clinical, and survival data were collected from the medical record. Diagnostic abdominal CT images were used to measure body composition with Slice-O-Matic software. Skeletal muscle area and radiodensity, as well as intramuscular, subcutaneous and intra-abodominal adipose tissue area and radiodensity were measured at baseline and at the last scan prior to death/last visit. Total body skeletal muscle and adipose indices were calculated from height using standardized equations. Tissue loss and rates of loss were calculated. Descriptive statistics were performed. Additional analyses, including rates of sarcopenia and sarcopenic obesity, relationship of cachexia to tumor response and survival, sex-specific measures, and comparison of phenotype frequencies with the FOLFIRINOX study are in progress.

Results: Interim analysis of the first 100 patients in this study demonstrates that most patients lost adipose tissue, muscle or both, although some gained. Cachexia prevalence was 72% overall, where cachexia is defined as any muscle loss. Intramuscular adipose loss was observed in 65% of patients. In contrast, 84% of patients lost visceral fat, and 85% subcutaneous fat. Overall, 22 patients gained >2.5% skeletal muscle, and 17 gained >5% visceral or subcutaneous adipose tissue. Overall, these 100 patients experienced mean skeletal muscle change of -5.54% (SD: 10.68%, range +20.4% to -33.41%, $P<0.0001$ one-sample T-test), visceral adipose change of -30.84% (SD: 48.08%, range +289% gain to -96.18%, $P<0.0001$), and subcutaneous adipose change of -32.53% (SD: 37.32%, range 167% to -98.57%, $P<0.0001$). Further statistical analyses are pending.

Conclusions: These results are consistent with literature estimates of rates of cachexia in PDAC near 85%, but demonstrate considerable patient variability in cachexia presentation. Further analyses of these data including comparisons of cachexia measurements with survival, tumor response, and results in patients on FOLFIRINOX will be presented.

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

POSTER #101

BODY COMPOSITION CHANGES AND CACHEXIA PHENOTYPES IN PATIENTS WITH PANCREATIC ADENOCARCINOMA ON GEMCITABINE/NAB-PACLITAXEL CHEMOTHERAPY

Andrew Young[1], Safi Shahda[1,3,4], Kristen Link[1], Leonidas Koniaris[2,3,4], Teresa Zimmers[2,3,5,4]

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Methods: This was a retrospective study of up to 141 patients with advanced PDAC treated with first-line gemcitabine/nab-paclitaxel at Indiana University. Demographic, clinical, and survival data were collected from the medical record. Diagnostic abdominal CT images were used to measure body composition with Slice-O-Matic software. Skeletal muscle area and radiodensity, as well as intramuscular, subcutaneous and intra-abodominal adipose tissue area and radiodensity were measured at baseline and at the last scan prior to death/last visit. Total body skeletal muscle and adipose indices were calculated from height using standardized equations. Tissue loss and rates of loss were calculated. Descriptive statistics were performed. Additional analyses, including rates of sarcopenia and sarcopenic obesity, relationship of cachexia to tumor response and survival, sex-specific measures, and comparison of phenotype frequencies with the FOLFIRINOX study are in progress.

Results: Interim analysis of the first 100 patients in this study demonstrates that most patients lost adipose tissue, muscle or both, although some gained. Cachexia prevalence was 72% overall, where cachexia is defined as any muscle loss. Intramuscular adipose loss was observed in 65% of patients. In contrast, 84% of patients lost visceral fat, and 85% subcutaneous fat. Overall, 22 patients gained >2.5% skeletal muscle, and 17 gained >5% visceral or subcutaneous adipose tissue. Overall, these 100 patients experienced mean skeletal muscle change of -5.54% (SD: 10.68%, range +20.4% to -33.41%, $P<0.0001$ one-sample T-test), visceral adipose change of -30.84% (SD: 48.08%, range +289% gain to -96.18%, $P<0.0001$), and subcutaneous adipose change of -32.53% (SD: 37.32%, range 167% to -98.57%, $P<0.0001$). Further statistical analyses are pending.

Conclusions: These results are consistent with literature estimates of rates of cachexia in PDAC near 85%, but demonstrate considerable patient variability in cachexia presentation. Further analyses of these data including comparisons of cachexia measurements with survival, tumor response, and results in patients on FOLFIRINOX will be presented.

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

POSTER #102

EVALUATING LOCAL SKIN HEATING AS POTENTIAL EARLY DETECTION METHOD FOR SMALL-FIBER NEUROPATHY DURING TAXOL®

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Background: Recent pre-clinical data suggest that damage to small-fiber nerves may play a role in the symptoms of *taxane-induced peripheral neuropathy* (TIPN) breast cancer survivors (BCS) develop during cancer treatment. However, methods for evaluating small-fiber neuropathy are limited, making it difficult to determine what role, if any, changes in small-fiber nerves play in this toxicity. Recently, a technique for detecting changes to small-fiber nerves has been developed. The technique, known as *local skin heating,* uses the neurogenic vasodilation marker for small-fiber nerve response. Research suggests that local skin heating may be a useful screening tool for small-fiber neuropathy in patients with diabetes; however, to date, local skin heating has not been tested as a screening tool for small-fiber TIPN in BCS receiving Taxol®.

Purpose: (1) To determine if local skin heating can detect signs of small-fiber TIPN in BCS during the first six weeks of Taxol®; (2) To determine if the severity of BCS' self-reported TIPN symptoms would be correlated with small-fiber nerve function, assessed using the size of axon reflexes/flares (both markers for small-fiber nerve function) in the toe during skin heating.

Methods: Design: Prospective, non-randomized, observational. Sample: N = 9 BCS with first-time, non-metastatic breast cancer receiving weekly or bi-weekly Taxol® and N = 20 healthy female controls (HCs). BCS were recruited from two local breast clinics. Data for both groups were collected at three time-points that took place in a six-week period: *Time 1* (day 0, pre-Taxol®), *Time 2* (day 14), and *Time 3* (day 42). Local skin heating was performed in the palmar toe surface using a validated 40-minute skin heating protocol. Axon reflex size was measured using Laser Doppler Flowmetry. Axon flare size was measured using Full-Field Laser Perfusion Imaging. Self-reported TIPN symptoms were measured using the 5-item short form of the Total Neuropathy Score (Reduced Version).

Results: There was a significant difference in axon reflexes for BCS at Time 3, indicating potential signs of small-fiber TIPN after 6 weeks of Taxol®. Contrary to expectation, axon reflexes were *larger* for BCS after six weeks of Taxol® than HCs ($p < .043$), suggesting that early exposure to Taxol® may be associated with an *increase* in small-fiber nerve function similar to that observed in pre-clinical studies. The severity of clinical TIPN symptoms were not correlated with axon reflex/flares size at the same time point.

Conclusions: Local skin heating may be a potential early detection method for small-fiber TIPN in BCS receiving Taxol®. Additional studies are needed to validate these findings and determine whether early exposure to Taxol® is associated with an increase in small-fiber nerve function, as suggested by the data.

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

POSTER #103

PHASE I STUDY OF THE HDAC INHIBITOR PANOBINOSTAT IN COMBINATION WITH THE MTOR INHIBITOR EVEROLIMUS IN PATIENTS WITH ADVANCED CLEAR CELL RENAL CELL CARCINOMA

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Background

Histone deacetylase (HDAC) inhibitors have been shown to promote the anti-tumor properties of mTOR inhibitors in pre-clinical studies. In this phase 1 trial we assessed the safety, tolerability, and recommended phase II dosing of the HDAC inhibitor panobinostat in combination with the mTOR inhibitor everolimus in patients with advanced clear cell renal cell carcinoma. We additionally evaluated the expression of microRNA 605 (miR-605) in serum samples obtained from trial participants as aberrant miR-605 expression has been implicated in carcinogenesis through modulation of the p53/Mdm2 axis.

Hypothesis

Panobinostat/everolimus combination therapy will be well-tolerated and have promising preliminary efficacy. There will be differential expression of miR-605 that correlates with treatment response.

Methods

Phase 1:

We used a rule-based, simultaneous dose escalation design to assess the primary objectives: safety, tolerability, and determination of the maximum tolerated dose (MTD). The secondary endpoint was evaluation of preliminary evidence of efficacy (Kaplan-Meier estimate).

miR-605:

miR-605 levels were measured at cycle 1/day 1 (C1D1), C2D1, and at time of progression. MicroRNA was extracted using the Qiagen miRNeasy mini-kit and quantified with spectrophotometry. Utilizing RT- and Q-PCR, miR-605 was isolated and amplified. The delta Ct method was used to calculate the relative expression of miR-605 using U6B as an endogenous control. Statistical analysis was completed with the Wilcoxon signed-rank and Mann-Whitney U tests.

Results

Phase 1:

21 patients were evaluable for toxicity and efficacy. There were 3 dose-limiting toxicities (DLTs): grade 4 thrombocytopenia (TCP), grade 3 TCP, and grade 3 neutropenia. Everolimus 5 mg PO daily and panobinostat 10 mg PO 3 times weekly (weeks 1 & 2) given in 21-day cycles was the recommended phase II dose based on the MTD of these agents. While 13 patients had stable disease, there were no objective responses. Six-month progression-free survival (PFS) was 31% with a median PFS of 4.1 months (95% confidence internal [CI]; 2.0 – 7.1).

miR-605:

13 patients had serum samples available for analysis. 8 patients attained stable disease (SD), whereas 5 patients progressed (PD) without clinical benefit. There was higher baseline expression of miR-605 in patients with PD vs those with SD (p=0.0112). PD patients' miR-605 levels decreased after the 1st cycle (p=0.0245), while SD patients' miR-605 levels increased (p=0.0179). The miR-605 levels of SD patients decreased at time of progression of disease (p=0.0250).

Conclusion

A safe and tolerable dosing regimen was established for combination panobinostat/everolimus therapy with myelosuppression as the major DLT. There were no objective responses to therapy, and PFS was similar to that seen with everolimus monotherapy. There was differential expression of miR-605 that correlated with treatment response, perhaps in relation to upstream effects of mTOR inhibition and/or p53 mutational status.

Translational/Clinical Research ***Internal Medicine Resident***

POSTER #104

NEUROLOGIC IMPAIRMENTS FROM PEDIATRIC LOW-GRADE GLIOMA BY TUMOR LOCATION AND TIMING OF DIAGNOSIS

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Purpose: To better characterizethe neurologic outcomes of low-grade gliomas (LGGs) according to tumor location and duration of presenting symptoms in children.

Methods: We retrospectively reviewed neurologic impairments in 246 pediatric patients with LGGs (88 with optic pathway and midline tumors, 56 with posterior fossa tumors, 52 with cerebral hemisphere tumors, 35 with brainstem tumors and 15 with spinal cord tumors) who were treated at St. Jude Children's Research Hospital between 1995 and 2005. We compared neurologic impairments (defined by Common Terminology Criteria for Adverse Events, version 4.03) by tumor location and prediagnosis symptom interval (PSI) (=¿3 months or <¿3 months) at first and last patient visits.

Results: The median age of diagnosis was 7.1 years; median PSI was 2.1 months; and median time to last follow-up was 11.6 years. LGGs in the cerebral hemispheres resulted in significantly fewer neurologic impairments, compared with that of other locations at baseline (P¿<¿0.001) and at last follow-up (P¿<¿0.001). In all patients, PSIs greater than 3 months resulted in a significantly higher incidence of ataxia and dysmetria at last follow-up (41.6%, P¿=¿0.003). Greater PSI was also significantly associated with worsening lower extremity motor weakness from cerebral hemisphere tumors; dysmetria from optic pathway and midline tumors; eye and visual dysfunction from posterior fossa tumors; and ear and vestibular disturbances from brainstem tumors (P¿=¿0.05).

Conclusion: Neurologic impairment in pediatric LGGs varies by tumor location, and PSIs greater than 3 months affect some functionally important neurologic outcomes.

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

POSTER #105

THE IMPACT OF INSURANCE STATUS ON DISPARITIES IN TIME TO SURGERY AMONG NON-METASTATIC BREAST CANCER PATIENTS IN INDIANA

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Introduction: Timeliness of care among breast cancer patients has been shown to affect overall and disease specific mortality. The objective of this study is to understand the implications of insurance status at diagnosis on time to surgery (TTS) among breast cancer patients in Indiana.

Methods: The Indiana cancer registry and the Indiana Network for Patient Care were queried for women ages 18-90 with stage 0-III breast cancer. The timeframe from biopsy proven diagnosis of breast cancer to first definitive surgery was evaluated. Surgical delay was defined as TTS > 60 days. Insurance groups were compared with bivariate analysis. A multivariable logistic regression model was used to evaluate the probability of surgical delay.

Results: The study included 9156 patients with 924 (10.1%) experiencing surgical delay. The median time to surgery was 29 days (IQR 20-43) and varied by insurance type—uninsured 24.5 days (IQR 17-36), Medicaid 33 days (IQR 21-51), private 29 days (IQR 20-44) and Medicare 28 days (IQR 20-42) (p=0.000). Medicaid OR 1.8 (95% CI 1.3, 2.5) (p=0.000), black race OR 2.7 (95%CI 2.1, 3.5) (P=0.000), =3 comorbidities OR 2.5 (95%CI 1.4, 4.4) (p=0.001), mastectomy OR 1.7 (95%CI 1.5, 2.1) (p=0.000) and a different facility of biopsy and first definitive surgery OR 1.8 (95%CI 1.5, 2.3) (p=0.000) were associated with surgical delay.

Conclusion: Indiana Medicaid breast cancer patients are the most likely insurance group to experience surgical delay. The lower TTS for other insurance groups, compared to Medicaid, suggests that breast cancer patients with Medicaid experience disproportionate barriers in access to timely definitive surgical treatment. As state legislative efforts evolve on enrollment of the Medicaid population further studies are needed to evaluate how treatment delays affect clinical outcomes.

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

POSTER #106

PACLITAXEL DOSE IN IPSC-GENERATED SENSORY NEURONS FOR EX VIVO MODELING OF TAXANE-INDUCED PERIPHERAL NEUROPATHY

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Paclitaxel is an FDA approved taxane chemotherapy for a variety of cancers including breast, lung, and ovarian cancer. Paclitaxel is one of the most widely used taxane therapies due to the significantly improved disease-specific outcomes of the treatment. However, taxane-induced peripheral neuropathy (TIPN) is the most common dose limiting side effect of paclitaxel and can be potentially irreversible. The exact mechanism of TIPN remains unclear although a few clinical risk factors such as race, age, body mass index (BMI), and comorbidities including diabetes mellitus have been established. The occurrence variation of TIPN among patients indicates that genetic factors play a predisposition role in the development of TIPN. Previous translational models of TIPN have relied on primary neurons derived from animal models, which do not completely recapitulate the genetics of a human model. Human induced pluripotent stem cells (iPSCs) offer an excellent route to generate human neurons *ex vivo*. Additionally, iPSCs can be generated from fresh patient blood samples, which will allow for a truly personalized cell model of TIPN. Using neurons derived from iPSCs to investigate taxane-induced neuronal damage ex vivo to model clinical TIPN has been reported, however, these neurons were exposed to a large range of paclitaxel doses. In this study, we aim to establish an optimal *ex vivo* dose of paclitaxel to best assess neuronal damage in our translational model of TIPN. In future work we will use this paclitaxel dose to study taxane-induced neuronal damage in clinical patient-derived iPSCs-neurons to explore genetic predisposition in the development of TIPN.

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

POSTER #107

ICOSL+ PLASMACYTOID DENDRITIC CELLS AS BIOMARKER AND INDUCER OF GVHD

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Graft-versus-host disease of the gastrointestinal tract (GI-GVHD) is a major cause of death post allogeneic hematopoietic cell transplant (allo-HCT). We recently identified a novel $CD4^+CD146^+CCR5^+$ T cell population increased in the blood of GI-GVHD patients (Li et al., JCI Insight, 2016). This T cell population coexpressed IFN-¿ and IL-17, the pathogenic T helper (Th) 17 phenotype, which was induced by Inducible COStimulator (ICOS). These data suggested an essential role of ICOS and its ligand, ICOSL, in the Th17-prone $CD146^+CCR5^+$ T cells generation during GI-GVHD. Thus, we next analyzed ICOSL expression on donor blood dendritic cells (DCs) [$HLA\text{-}DR^+CD11c^+$ conventional (c)DCs, and $HLA\text{-}DR^+CD123^+$ plasmacytoid DCs (p)DCs] in the same cohort of patients. We showed high ICOSL expression on pDCs but not cDCs in GI-GVHD patients (n=64) compared to patients without GI-GVHD (n=39) or non-GVHD enteritis (n=22) (28%, 5% and 7% respectively, all $p< 0.0001$).

To evaluate the role of ICOSL on donor DCs, we used a well-established clinically relevant model of major histocompatibility complex (MHC)-mismatch HCT in which cells from C57BL/6j ($H\text{-}2^b$) mice are transferred to lethally irradiated BALB/c ($H\text{-}2^d$). We used $5x10^6$ bone marrow (BM) cells containing DCs that are either ICOSL-/- or wild-type (WT), plus $1x10^6$ WT donor T cells in both groups. Transplantation of ICOSL-/- donor BM resulted in a better survival compared to the recipients transplanted with WT BM ($p<0.0001$). The growth factor fms-related tyrosine kinase 3 ligand (Flt3-L) promotes the development of pDCs (Pulendran et al., Journal of Immunology, 1997), and signal transducer and activator of transcription 3 (STAT3) is required for Flt3-L dependent DCs differentiation (Laouar et al., Immunity, 2003). Using the same model as above, transplantation of $STAT3\text{-}/\text{-}^{CD11cCre}$ BM did not show any difference in survival. Altogether, these data suggest that ICOSL in donor BM is crucial to the pathogenesis of GI-GVHD, but STAT3 is not essential. Next, we analyzed the recipients' intestinal cells at day 10 post-HCT. First, we looked at pDCs ($CD11b^-CD11c^+B220^+CD103^+$) and found lower frequencies of infiltrating pDCs in recipients of ICOSL-/- BM as compared to recipients of WT BM (18% vs. 35%). Second, we analyzed the intestinal T cells and found lower frequencies of proliferating $Ki67^+$ Th1 (32.2% vs. 48.7%, $p<0.05$) and Th17 cells (18.6% vs 32.2%, $p<0.05$) in ICOSL-/- BM recipients compared as WT BM recipients. Last, we evaluated the levels of Flt3-L and found decreased Flt3-L levels in plasma collected at day 3 from ICOSL-/- BM recipients compared to the mice that received WT BM (642 pg/ml vs 1184 pg/ml, $p< 0.05$).

In conclusion, our findings suggest that 1) increased frequencies of ICOSL+ pDCs can serve as an early biomarker of GI-GVHD, 2) ICOSL on donor pDCs stimulates Th17 prone $CD146^+CCR5^+$ T cells and promotes GI-GVHD in a murine model. Future directions will use ICOS-ICOSL blockade to ameliorate GVHD in an experimental murine and in a human PBMCs xenogeneic model.

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

POSTER #108

PARENTAL EXPERIENCES OF CHILD PARTICIPATION IN A PHASE I PEDIATRIC ONCOLOGY CLINICAL TRIAL: "WE DON'T HAVE TIME TO WASTE"

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Purpose: Phase I clinical trials (P1Ts) are essential to developing new anti-cancer therapies for children with cancer, yet they raise complex ethical concerns about balancing the need for this research with the well-being of participating children. To address these concerns, it is important to understand the experiences of children with cancer and their parents in these trials. Although there are numerous studies on the P1T consenting process, no studies have been done with children with cancer or their parents on the experience of actually participating in a P1T. The purpose of this study was to describe the experience of P1T participation and its meaning from the perspective of parents of children with cancer.

Methods: This was a descriptive, empirical phenomenology study of 11 parents' experiences of their child's participation in a pediatric oncology P1T. Parents were recruited from two pediatric academic medical centers in the Midwest United States and national childhood cancer advocacy groups. Procedures included a demographic form, phenomenological unstructured interview, follow-up call, and extraction of data from the child's clinical trial record. Data were analyzed using an adapted Colaizzi method for empirical phenomenology.

Results: Pervasive throughout parents' descriptions of their lived experiences during P1T participation was a sense of running out of time to find an effective treatment for their child, and their need to use well the time they had with their child. Despite unique aspects of P1T participation, parents' experiences were not focused on the P1Ts themselves. Instead, parents were focused on their role and responsibilities as a parent, the specialness of their child, and their child's contending with aggressive high-risk cancer to survive. Parents' perceptions of their child's experiences reflected a sense of pride in their child and how their child dealt with the cancer and its treatments. What parents remembered following participation in a P1T reflected what stood out in the experience, and how parents managed the P1T experience. Particularly important aspects of the P1T experience included the connection with the clinicians who managed the child's care during the P1T, making the choice to continue trying to slow or stop the child's cancer by participating in a P1T, and being burdened by additional requirements when participating in a P1T.

Conclusions: Overall, while some concerns were raised regarding P1T experiences, parents did not regret their child participating in a P1T and would recommend P1Ts to other parents of children with cancer.

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

POSTER #109

ROLE OF SYSTEMIC THERAPY IN PLASMACYTOID UROTHELIAL CARCINOMA

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Background: Plasmacytoid urothelial carcinoma (PUC) is a rare variant histology where malignant cells resemble those of plasmacytoma. Majority of the patients (pts) are upstaged at the time of cystectomy and prognosis is extremely poor. Management remains controversial.

Methods: We retrospectively reviewed treatments and outcomes in pts with PUC seen at our institution between 1996 and 2017. The Kaplan Meier method was used to calculate overall survival.

Results: A total of 72 pts (59 male, 13 female) with a median age of 67 (range, 3690) were identified. Six pts presented with metastatic disease, whereas 66 were diagnosed with clinically localized PUC. Forty-five pts had upfront radical cystectomy (RC), 16 had neoadjuvant chemotherapy followed by RC (NAC-RC), and 11 elected for bladder preservation approaches. Upstaging was seen on postop pathology in 13 (81%) of pts who received NAC-RC. Only one pt had a pathologic complete response with pT0N0. Seventeen pts received adjuvant chemotherapy, 2 of which received NAC as well. Median overall survival (OS) for all pts from time of initial diagnosis was 17.7 months (mo), (stage IIII vs stage IV: 21.0 vs 10.4 mo, p = 0.053). Median OS was 12.6 mo from time of cystectomy for patients undergoing RC. Median OS for pts who received NAC was 15.9 mo, compared to 82.8 mo in pts who received adjuvant chemotherapy and 10.1 mo in pts undergoing cystectomy alone (p = 0.086). Median recurrence free survival (RFS) was 13.9 mo. Median RFS for pts who received NAC was 12.9 mo, compared to 15.7 mo in pts who received adjuvant chemotherapy and 13.9 mo in pts undergoing cystectomy alone (p = 0.073). Median OS in 9 pts with pure (100%) PUC compared to mixed plasmacytoid variants (PCV) was 46.1 vs 15.9 mo (p = 0.503). Median RFS in pts with pure PU compared to mixed PCV was 34.3 vs 13.8 mo (p = 0.0102). Pts with < 75% PCV morphology had an OS of 11.6 mo compared to 21.4 mo in pts with =75% PCV morphology (p = 0.312).

Conclusions: PUC is an aggressive variant with overall poor outcomes. NAC-RC appeared to result in poor outcome while adjuvant chemotherapy appeared to trend toward better clinical outcome. The amount of PCV morphology did not appear to have an effect on outcome.

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

POSTER #110

LONG-TERM SURVIVAL AND LOCAL CONTROL OUTCOMES IN OLIGOMETASTATIC COLORECTAL CANCER TREATED WITH LIVER STEREOTACTIC BODY RADIATION THERAPY LONG-TERM SURVIVAL AND LOCAL CONTROL OUTCOMES IN OLIGOMETASTATIC COLORECTAL CANCER

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Purpose/Objective(s):

The purpose of the present study was to evaluate clinical outcomes following stereotactic body radiation therapy (SBRT) in patients with liver metastases (LM) due to colorectal cancer (CRC).

Materials/Methods:

This was a single-institution retrospective study of patients included in a prospectively maintained institutional database. Outcome endpoints included overall survival (OS), local control (LC), progression-free survival (PFS), and extra- and intra-hepatic progression-free survival (EHPFS/IHPFS). Eligible patients had LM due to CRC, were treated with SBRT between 2007-2017, and had at least 3 months of follow up. We identified 55 patients that met these criteria; 13/55 (24%) of patients had prior partial hepatectomy. Median number of chemotherapy regimens prior to SBRT was 1 (range 0-3). The majority of patients (81.6%) had a solitary liver metastasis; 2 had extrahepatic disease at the time of SBRT. The maximum number of treated LM was 3. Median SBRT dose was 54 Gy (range 32-60 Gy) in 3 fractions (range 3-5), and median BED_{10} was 151.2 Gy. Median tumor diameter was 2.8 cm (range 0.4-5.6). All survival times were calculated from the start date of SBRT. Kaplan-Meier curves were used to estimate actuarial survival times, and Cox regression was used for multivariable analysis (MVA) of predictors of overall survival.

Results:

At a median follow up of 44.5 mos (range 3-96), 24/55 patients (43.6%) of patients have died. Estimated median OS was not reached; estimated mean OS was 61.7 months (95% CI 51.7-71.7), and actuarial 5-year OS estimate was 21%. Median PFS was 13.0 months (95% CI 2.6-23.4); median IHPFS was 26 months (95% CI 15.4-36.4), longer than the estimated median EHPFS of 18 months (95% CI 9.4-26.6). The crude overall rate of hepatic progression was 60.0% (33/55), while the crude rate of extrahepatic progression was 65.4% (36/55). Crude local control was 76% (13/55). Median local control time was significantly longer in patients with higher biologic equivalent dose (BED); median LC was 24 mos vs not reached in patients with BED_{10} < vs > 150, p = 0.002. OS was also improved in patients with BED_{10} > 150 (median 32 mos vs not reached and 5-yr OS 29 vs 58% in patients with BED_{10} < vs > 150, p = 0.03). Cox MVA showed that early local failure was associated with poorer OS (p = 0.004); higher baseline CEA at the time of treatment was also associated with a trend towards worse OS (p = 0.063).

Conclusion:

SBRT is very effective in the management of hepatic oligometastases and was associated with an estimated mean OS of over 5 years in selected patients. In this series, 56% of patients who underwent liver SBRT for oligometastatic CRC remain alive after a median follow up of nearly 4 years (median 44.5 months). Overall

LC was excellent and was strongly associated with higher BED. Early local progression was also associated with inferior OS, supporting efforts to achieve as high a BED as possible in SBRT plans for oligometastatic CRC.

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

POSTER #111

A PILOT STUDY TO ASSESS THE ACCURACY OF "INTUITION" IN ANALYZING POST CHEMOTHERAPY (CHEMO) RESIDUAL RETROPERITONEAL (RP) MASSES IN PATIENTS (PTS) WITH NON SEMINOMATOUS GERM CELL TUMOR (NSCGCT)

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Background

About half of the pts operated for residual RP mass after chemo for NSGCT do not benefit from surgery because the residual mass contains only necrosis. We sought to assess the accuracy of "intuition" or experienced guess by urologists at a large tertiary referral center for identifying malignant tumors (teratoma and/or viable cancer cells) after primary chemo for disseminated NSGCT.

Methods

We included pts with persistent RP mass after chemo who were referred to the urology clinic for assessment for post chemo RP lymph node dissection (PC RPLND). All pts had normal tumor markers (serum HCG and AFP) after completion of chemo and at the time of analysis. The urologists looked at clinical data – pt history, orchiectomy pathology, post chemo CT scans, tumor markers and made an intuitional analysis of the residual mass as teratoma, necrosis or malignancy, which was stored in an encrypted format. After pts had RPLND, their histology was compared to the intuition analyses.

Results:

Pt characteristics are summarized in Table 1. The urologists' intuition matched the final pathology in 37/53 (70%) cases. Table 2 shows number of correct predictions. Table 3 shows pathology of incorrect predictions. There was a trend towards an interaction of residual mass size and urologist intuition to correctly predict histology, but this was not statistically significant (p=0.57).

Conclusion:

Urologists at a tertiary care center with experience of treating germ cell tumor pts can predict histology reasonably well. Future directions will be implemented towards combining this with radiology and potential molecular assessments to reduce surgeries in pts with necrosis.

TABLE 1

CHARACTERISTICS	**Number (%)**
Median Age (range)	29 (25-37)
Teratoma in orchiectomy	
· Yes	32 (62)
· No	20 (38)
Chemotherapy regimens	

· BEPx3	16 (30)
· EPx4	9 (17)
· BEPx4	15 (28)
· Combination of BEP/EP	5 (9)
· Combination of BEP/VIP	2 (4)
· VeIP	1 (2)
· HDCT	1 (2)
· VIP	4 (8)

TABLE 2

	RPLND histology	**Correct prediction (%)**
Cancer	2	0(0)
Teratoma	38	30(79)
Necrosis	13	7(53.9)

TABLE 3Pathologic correlation of Urologists' intuition

	Urologist intuition	**RPLND histology**
Cancer	0	-
Teratoma	38	30 Teratoma, 6 Necrosis, 2 Cancer
Necrosis	15	7 Necrosis, 8 Teratoma

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

POSTER #112

DISSEMINATION OF BREAST CANCER KNOWLEDGE AND EXPERTISE FROM NCI-CCC TUMOR BOARDS WITH COMMUNITY ONCOLOGISTS USING AN ONLINE PLATFORM

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Introduction:While tumor boards (TB) at National Cancer Institute Designated Comprehensive Cancer Centers (NCI-CCC) are an important source of multidisciplinary education and expert knowledge, this knowledge is not systematically documented and made accessible to oncologists in the community. Using an online oncologist-only question and answer (Q&A) website, we sought to document expert insights from TBs at NCI-CCCs to provide educational benefit to the oncology community.

Methods: We designed a process with oncologists at the MD Anderson, University of Pittsburgh, UCLA, University of Utah, and Yale Cancer Centers to document and share discussions from breast cancer TBs focused on areas of controversy and practice variation on theMednet.org, a social Q&A website of over 5,300 US oncologists. Faculty members translated TB discussions into concise non-case based Q&A on theMednet. Answers were peer reviewed by experts at other institutions, indexed and stored for easy search retrieval, and disseminated in a weekly email newsletter to registered medical oncologists. Engagement was measured in Q&A views and helpful and agree votes. Reach was measured by the number of unique oncologists viewing Q&A. Answers were analyzed for reference sources.

Results: Between 12/2016 and 1/2018, 55 answers to 50 questions were posted from 5 NCI-CCCs and shared with 1,994 medical oncologists. Twenty-nine academic breast oncologists peer reviewed answers. Answers were viewed by 856 oncologists at 550 institutions in 49 states. This included 469 community practices. All questions were focused on topics not addressed by NCCN or ASCO guidelines or on controversy in guidelines. Forty-seven percent of answers cited published research and 62% cited clinical experience.The median time from research publication date to Q&A on theMednet was 10.5 months (range 0.1-96).

Conclusion: Through an online Q&A platform, experts at NCI-CCCs document, peer-review, and disseminate clinical experience, new research, and important knowledge outside the walls of academic cancer centers to oncologists across the US. Access to up to date, expert knowledge can significantly impact patient care in community practices.

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

POSTER #113

OUTCOMES OF PEDIATRIC ONCOLOGY AND HEMATOPOIETIC CELL TRANSPLANT PATIENTS RECEIVING ECMO

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Objective: There is much debate over the utilization of extracorporeal membrane oxygenation (ECMO) in pediatric patients with an underlying oncologic process or who have undergone hematopoietic cell transplant (HCT). We hypothesized that these patients have a higher mortality rate, more bleeding complications, more blood product utilization and a higher rate of new infections than the general critically ill pediatric population supported with ECMO.

Design: Retrospective chart review.

Setting: Single center, quaternary care pediatric hospital.

Patients: Patients supported on ECMO in the pediatric intensive care unit from 2011-2016.

Interventions: None.

Methods and Main Results: Patients were categorized into the oncology/HCT or non-oncology/HCT. Continuous variables are displayed as medians with interquartile ranges (IQR) and were compared using the Mann-Whitney U test. Categorical variables are reported as frequencies and percentages and were compared with chi squared or Fisher's Exact tests. A total of 38 ECMO patients met inclusion criteria. Of these, seven were oncology/HCT patients. Those in the oncology/HCT group had lower platelets at the start of ECMO ($p=0.02$). ECMO survival was significantly lower in this group (28.6% vs 77.4%, $p=0.02$). Odds ratio for mortality was 8.6 (95% CI: 1.4, 54.2). Bleeding complications and new infections did not differ significantly. The oncology/HCT group received more platelets (15.9 ml/kg/day (8.4-36.7) vs. 7.9 ml/kg/day (3.2-21.9)) and fresh frozen plasma (14.0 ml/kg/day (3-15.7) vs. 1.8 ml/kg/day (0.5-5.9)).

Conclusions: Oncology and HCT patients had a significantly higher ECMO mortality and received more blood products while on ECMO than the general population despite similar pre-ECMO characteristics. Based on this data, overall prognosis and function of the bone marrow, particularly in terms of platelet recovery, should be significant factors for consideration when determining sustainability for ECMO.

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

POSTER #114

DEVELOPMENT OF TRANSAMINITIS IN A PATIENT WITH CHRONIC LYMPHOCYTIC LEUKEMIA ON ACTIVE TREATMENT: A CASE REPORT

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Chronic lymphocytic leukemia (CLL), one of the most common leukemias in the United States, presents at a median age of 65 and is typically classified as an indolent disease with classic “smudge cells” on peripheral smear. The United States uses the Rai staging system, with Rai stages III/IV being considered high risk disease with a 50% survival at 5 years. Poor prognostic indicators include bone marrow infiltration, del(17p), del(11q), beta-2 microglobulin, CD38+, ZAP-70 elevations, lymphocytic doubling time less than 12 months, and the presence of transaminitis. CLL involvement of the GI tract is a rare clinical manifestation, affecting only 3% of those diagnosed. In addition, 5% of untreated patients will have an elevation of at least one liver function test. We report a case of a 41-year-old male with CLL Rai Stage I (+Zap-70, +CD38) who was found to have progressive disease. He had a past medical history of DM II, CKD stage III, CAD with LAD stent placement and recurrent UTI. The patient had been treated with two standard lines of therapy when he developed extensive bone marrow involvement, clinically consistent with Rai Stage IV CLL. He was started on Ibrutinib, a selective inhibitor of Bruton’s tyrosine kinase as a third-line agent. At the time, this medication was recently approved for CLL in patients who had one prior therapy with Rai Stage IV CLL. After one week of initiation of Ibrutinib, the patient developed abnormally increased liver function tests: AST and alkaline phosphatase > 3x ULN and ALT >18x ULN. Patient was mildly symptomatic with sporadic abdominal pain. With subsequent breaks in his cycles of therapy and dose reductions, these liver tests normalized. Ibrutinib induced transaminitis is an atypical side effect that has only been previously documented in two case studies. To complicate the picture further, the patient was found to have CLL involvement of the liver upon biopsy. This case explores the workup and differentials of a rare complication of CLL, elevated liver enzymes, in the setting of initiating a novel targeted therapy.

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

POSTER #115

IDENTIFICATION OF CIRCULATING PROTEIN BIOMARKERS FOR PANCREATIC CANCER CACHEXIA

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Introduction: Cancer cachexia is a multifactorial paraneoplastic syndrome with more than 80% of patients with pancreatic ductal adenocarcinoma (PDAC) suffering from this debilitating disease. Although considerable progress has been made in understanding the pathophysiology of cachexia using various experimental models of cachexia, translation of these findings in human cancer cachexia remains limited. Therefore, our aim was to investigate the pathophysiology of cachexia in pancreatic cancer by quantifying protein levels from serum samples of patients with PDAC.

Methods: Serum from 29 patients with PDAC were profiled via Somascan, an aptamer-based platform that quantifies relative levels of 1249 proteins. After quality control processing, quantile normalized values were used for downstream analyses. Partial Spearman's rank-order correlation, adjusting for age and sex, identified proteins correlated with cancer associated weight loss (CAWL) category (an ordinal classification of history of weight loss and BMI), skeletal muscle index (SMI), total adipose index (TAI), and skeletal muscle density (SMD). Proteins with effect size =0.5 and $p<0.05$ were further analyzed. Functional enrichment analysis was performed via DAVID to identify clusters with enrichment value of =1.5 and $p<0.05$. Further, co-expression analysis among all 1249 proteins was carried out using pairwise Spearman's correlation to identify co-regulated protein networks. Among those, we have focused initially on the IL-6 pathway given its central role in PDAC cachexia. Co-expression networks were identified using Ingenuity Pathway Analysis.

Results: A total of 111 proteins correlated with clinical measures of cachexia (48 with CAWL, 19 with SMI, 14 with SMD, and 30 with TAI). LYVE1, a homolog of CD44 implicated in tumor metastasis, was the top CAWL-associated protein ($r= 0.67$, $p=0.0001$). Other highly correlated proteins included coagulation factors C7, F2, and C5, the lymphocyte surface antigen LY9, and IL1RL1, suggesting inflammation. Other proteins such as INHBA, MSTN, PIK3R1 were also correlated with CAWL. Many proteins correlated with CAWL were also correlated with SMI, SMD and TAI. This is anticipated because muscle volume and fat volume both decline with increasing CAWL. Consistent with known features of cachexia, enriched pathways included the immune response, cytokine signaling pathways, and proteolysis. Serum IL-6 and STAT3 levels correlated with each other and each with history of weight loss. Protein co-expression analysis of IL-6 and related proteins identified networks involved in glucocorticoid signaling, PI3K/AKT signaling, and mTOR signaling, as well as Th1 and Th2 pathways, and T cell and B cell receptor signaling.

Conclusion: We have identified protein biomarkers for PDAC cachexia, including several known modulators as well as some previously unidentified markers. Identifying these pathways in blood is promising because

blood sampling can be used as a minimally invasive method to study the human biology of cachexia. Pathway analysis should provide novel insights into molecular underpinnings of fat and muscle loss in PDAC.

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

POSTER #116

MECHANICAL SIGNALS RETAIN MUSCULOSKELETAL ENDPOINTS WHILE SUPPRESSING ADIPOSITY IN A MURINE MODEL OF COMPLETE ESTROGEN DEPRIVATION

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Post-menopausal, estrogen (E_2) receptor-positive breast cancer patients treated with aromatase inhibitor (AI) experience deleterious effects to bone and muscle, while creating an environment permissive to accrual of adipose. Pharmacological agents prescribed to inhibit osteoclast-mediated resorption are effective in preventing bone loss but may have adverse effects. Low intensity vibrations (LIV), a low magnitude mechanical signal that accelerates in the high-frequency domain, mimicking dynamic forces of skeletal muscle contractility on bone, have been shown to safely preserve bone in two cancer models, reduce adipogenesis in mice challenged by a high fat diet, and upregulate muscle-bound satellite cells in ovariectomied (OVX) mice. Therefore, we hypothesized that LIV could preserve bone mass and muscle strength following complete E_2-deprivation. Twenty weight-matched, 4w old female C57BL/6 mice were treated with LIV (90Hz, 0.35g, 1x/d) or untreated (CTL) for 4w. Complete E_2-deprivation by OVX and maintained via daily treatment with AI (letrozole) followed for all mice, while LIV-mice continued on 24w of additional LIV treatment. Longitudinal body composition measurements for lean mass, fat mass and bone mineral density (BMD) were quantified by dual energy x-ray absorptiometry (DXA) every 3w. Total fat mass was significantly lower ($p<0.0001$) in LIV-mice throughout the experiment as compared to CTL-mice, a 63% difference by week-28. Conversely, quantification of whole body lean tissue mass showed a significant effect between groups, with LIV-mice bearing 7% ($p<0.03$) more lean mass at week-28. Functional grip strength measurements were significantly greater in LIV-treated mice across the study as compared to CTL ($p<0.04$). A glucose tolerance test at week-26 showed LIV-mice had greater serum blood glucose ($p=0.056$) than CTL, as quantified by the area-under-the curve. 24w post-OVX/AI induction, trends indicating reduced serum leptin were observed in LIV-mice. Using micro-computed tomography, a 22% ($p<0.05$) increase in trabecular bone volume fraction and a 52% ($p<0.01$) increase in connectivity density were observed in the L5 vertebrae of LIV-mice as compared to CTL-mice. Dynamic histomorphometry of fluorochrome-labeled femora demonstrated greater bone formation rates ($p<0.001$) and mineralizing surfaces ($p<0.001$) in LIV-treated mice relative to CTL. As assessed via DXA, no differences in BMD were observed between groups. While the contributions of OVX and daily AI injections resulted in reduced bone mass and muscle strength due to the absence of circulating estrogens, our findings suggest that LIV may be a novel means of protecting and/or increasing lean mass, reducing fat mass and improving bone endpoints as otherwise compromised by total E_2-deprivation.

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

POSTER #117

COMBINATION THERAPY IN PDAC INVOLVING BLOCKADE OF THE APE1/REF-1 SIGNALING PATHWAY: AN INVESTIGATION INTO DRUG SYNTHETIC LETHALITY AND ANTI-NEUROPATHY THERAPEUTIC APPROACH

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Pancreatic ductal adenocarcinoma (PDAC) is the 4th leading cause of cancer-related mortality in the US. Most patients present with advanced disease and ~93% die within five years, with most surviving less than six months. Combination therapies including Gemcitabine (Gemzar^{TM}) and sustained release, nab-paclitaxel (Abraxane^{TM}) and FOLFIRINOX (5-FU/leucovorin/irinotecan/oxaliplatin) offer modest improvement in survival, albeit at an increase in side effects including chemotherapy-induced peripheral neuropathy. Data is presented on Apurinic/apyrimidinic endonuclease/redox factor-1 (APE1/Ref-1 or APE1) and redox-specific APE1 inhibitor, APX3330 and its effects on tumor cell growth and sensory neuron function.

APE1 is a multifunctional protein involved in repairing DNA damage via endonuclease activity and in redox regulation of transcription factors such as HIF-1a, NFkB and STAT3. High expression levels of APE1 indicate decreased survival in PDAC as well as other cancers. Because APE1 is essential for cell viability, generation of APE1 knockout cell lines and determining a comprehensive list of genes regulated by APE1 has been difficult.

To circumvent this, we performed single cell RNA-Sequencing on PDAC cells following APE1 knockdown under normoxia and hypoxia to identify differentially expressed genes and further explore APE1's effects on HIF-1a and STAT3 signaling under both conditions. Proteomic analysis on PDAC cells following APE1 knockdown in normoxia and hypoxia revealed changes in signaling downstream of APE1, complementing the transcriptomic data and providing a more complete understanding of pathways affected by APE1.

We used the newly identified APE1 targets and pathways along with drug sensitivity data of cancer cell lines from the Cancer Cell Line Encyclopedia (CCLE) to generate potential combination therapies of FDA approved drugs and the APE1 redox inhibitor, APX3330 and next generation analogs. These combinations were tested using an *ex vivo* 3D tumor-stroma model system using patient derived cells from the tumor as well as cancer-associated fibroblasts. We identified synergy with agents such as Napabucasin and Entinostat.

We also tested APX3330 in combination with drugs that are part of PDAC standard of care. *In vivo* studies combining APX3330 with Gemcitabine showed significantly decreased tumor volume. Combining oxaliplatin (part of FOLFIRINOX) with APX3330 caused a significant reduction in oxaliplatin-induced DNA damage in sensory neurons from a KPC orthotopic graft model, without hindering its anti-cancer activity. With the phase I clinical trial for APX3330 underway (IND 125360), the potential for APE1 targeted therapy enhancing tumor efficacy while providing neuroprotective effects in the sensory neurons provides a win-win scenario.

POSTER #118

TUMOR-SPECIFIC GENOMIC ABERRATIONS DICTATE SYSTEMIC EFFECTS OF BREAST CANCER

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Functional limitation and sarcopenia with or without weight loss are the two major systemic effects observed in breast cancer patients. Cachexia, which is often debilitating and irreversible, is observed in ~25% of breast cancer patients. We recently utilized MMTV-PyMT mammary tumor model system to functionally characterize systemic effects of breast cancer. We observed specific aberrations in skeletal muscle of tumor-bearing mice with accompanying functional limitation and body composition changes. These cancer-induced systemic effects could be reversed partially by the pharmacologic agent DMAPT, which targets the signaling molecule NF-kB. It is unknown whether the degree of systemic effects and the type of molecular changes in skeletal muscle vary between breast cancer subtypes. Genome-wide comparative analyses of multiple transgenic mammary tumor models have suggested MMTV-PyMT model to represent the luminal B intrinsic subtype and MMTV-Neu model to represent Her2-amplified subtype. Through comparative analyses of these two models, we demonstrate that breast cancer subtypes have an influence on molecular changes in skeletal muscle. While reduced expression of skeletal muscle stem cell (MuSC) and aging-associated transcription factor Hoxa9, lower CD82+/CD54- MuSC subpopulation, and increased extracellular matrix deposition accompanied with lower grip strength and rotarod performance were observed in both models compared to control animals, reduced expression of another MuSC transcription factor Pax7 and metabolic pathway alterations involving mitochondrial dysfunction were observed only in MMTV-PyMT model. Pgc1b, which is involved in mitochondrial biogenesis, was specifically reduced in skeletal muscle of MMTV-PyMT mice. The majority of skeletal muscle changes in MMTV-PyMT mice suggested tumor-induced accelerated aging of skeletal muscle. We also observed distinct circulating cytokine profiles in two models. While cachexia-promoting TGFb2 and TNFa were upregulated in MMTV-PyMT mice, eosinophil-derived cachexia-associated eotaxin was upregulated in MMTV-Neu mice. These results suggest that cancer-specific genomic aberrations have an impact on the type of molecular changes in skeletal muscle. In addition, these results explain for the lack of clinical success of cachexia-targeted clinical trails as these trials did not integrate cancer genomics with symptom science to individualize therapies.

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

POSTER #119

RECIPROCAL ROLES OF ST2 AND RORγT EXPRESSION IN INTESTINAL CD4+ FOXP3+ REGULATORY T CELLS DURING INFLAMMATION

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$CD4^+$ $Foxp3^+$ Regulatory T cells (Tregs) play a protective role in intestinal inflammation, and expression of the membrane-bound form of STimulation-2 (ST2), the IL-33 receptor, on Tregs improve their suppressive function *in vivo* during intestinal inflammation. However, it is unknown by which mechanism. We studied the role of $ST2^+$Tregs in murine models of allogeneic hematopoietic cell transplantation (allo-HCT). To investigate the origin of $ST2^+$Tregs, we used the allografts in which $Foxp3^-$ conventional T cells (Tcons) and Tregs were sorted from different Foxp3 reporter mice (Tcons from $Foxp3^{RFP}$ mice, Tregs from $Foxp3^{GFP}$ mice) to establish a minor mismatch acute graft-versus-host disease (aGVHD) model (C57BL/6, H-2^b to C3H.SW, H-2^b). We found $ST2^+$ Tregs were expanded from donor natural Tregs during aGVHD and were predominantly localized in aGVHD target organs (intestine and liver), but not in the secondary lymphoid organs. We have shown before that $ST2^+$Tregs prevent the development of aGVHD in experimental model. Here we showed that absence of ST2 on donor Tregs decreased their proliferation (Ki67), activation (CD69, KLRG1), and function (LAG-3, IL-10) while infiltrating the inflamed intestine. Furthermore, transcriptome analysis of intestinal $ST2^{-/-}$ vs. WT Tregs from both major and minor mismatch aGVHD models showed, as expected, downregulation of Tregs markers (Foxp3, Ctla4, Il27, Icos, Il1rl1, Il2ra, Ahr) while conversely increased Rorc expression. Transplantation of Rorc deficient T cells led to increase frequency of $ST2^+$Tregs in the intestine and lower aGVHD score and mortality. This was due to a reduced frequency of sST2 producing T cells with an increased frequency of protective ST2 expressing T cells (Tregs, IL-4^+IL-10^+ Th2 cells) in the intestine. Adoptive transfer of donor polyclonal Tregs stimulated by IL-33 ($Treg_{IL\text{-}33}$) prevented aGVHD *in vivo* compared to Tregs cultured without IL-33. We also showed that the effect of IL-33 on Tregs can be inhibited by addition of IL-23/IL-17. All together, we conclude that ST2/IL-33 activation of both mouse and human Tregs may serve as a new T cell therapy in aGVHD.

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

POSTER #120

IMPACT OF SPIRITUAL WELL-BEING ON DEPRESSION, ANXIETY, AND GLOBAL HEALTH OF BREAST CANCER SURVIVORS WITH CLINICALLY SIGNIFICANT FEAR OF RECURRENCE

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Background: Fear of cancer recurrence (FCR) is a prevalent and persistent problem for approximately half of post-treatment breast cancer survivors (BCS). FCR is a top unmet supportive care need and has a negative impact on emotional and physical health for many BCS. Spirituality may play a significant role in coping with FCR. Examining the extent to which spiritual well-being influences depression, anxiety, and global health among BCS is the basis of this secondary analysis of data from a randomized pilot that tested 3 approaches to addressing FCR.

Methods: Eligible BCS (n=91) were diagnosed at an early stage (I-III), had completed curative treatment, and reported clinically-significant FCR (Fear of Cancer Recurrence Inventory [FCRI] Short Form score = 13). Participants were randomized to 1 of 3 interventions (6-week Acceptance and Commitment Therapy group; 6-week survivorship education group; usual care enhanced with survivorship readings). To determine the impact of spiritual well-being (FACIT-Sp) on depressive symptoms (PHQ-8), anxiety (GAD-7), cancer-related post-traumatic stress (IES-R), fear of recurrence (FCRI), physical and mental global health (PROMIS), vitality (SF-36 Vitality), and hope over time, participants completed measures of these constructs at baseline, 6 and 10 weeks, and 6 months. Analyses were performed using repeated measures, analyzing the change in outcomes from baseline to each of the follow-up time periods and adjusting for the baseline value, as well as assessing the effect of treatment group.

Results: FACIT-Sp was significantly associated with all of the clinical measures ($p<.05$). As spiritual well-being increased, negative clinical measures (PHQ-8, GAD-7, IES-R, and FCRI) decreased (regression coefficients: -1.72, -2.50, -4.14, and -0.22, respectively). Further, as spiritual well-being increased, positive clinical measures (global health physical, global health mental, vitality, and hope) increased (regression coefficients: 0.92, 1.70, 13.14, and 0.41 respectively). The three FACIT-Sp subscales (meaning, peace, and faith) were also associated in similar directions, although the effect sizes were smaller in every case. The only exception was that the faith sub-scale and IES-R were not significantly associated. Treatment group was not associated with any of the FACIT-Sp subscales.

Conclusions: We conclude that spiritual wellbeing may likely be associated with several emotional and spiritual distresses among adults dealing with fear of cancer recurrence. Findings suggest that oncology clinicians can enrich their care of survivors by supporting survivors in deepening their spiritual well-being.

Translational/Clinical Research ***Research Chaplain***

POSTER #121

APE1/REF-1 REDOX SIGNALING REGULATES HIF1A-MEDIATED CA9 EXPRESSION IN HYPOXIC PANCREATIC CANCER CELLS: COMBINATION TREATMENT IN PATIENT-DERIVED PANCREATIC TUMOR MODELS

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Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease characterized by aggressive metastasis and therapeutic resistance. Reactive stroma in PDAC tumors leads to fibrosis, inflammation, and hypoxia. Hypoxia signaling creates a more aggressive phenotype with increased potential for metastasis and decreased therapeutic efficacy. Carbonic anhydrase IX (CA9) functions as part of the cellular response to hypoxia by regulating intracellular pH to promote cell survival. Apurinic/Apyrimidinic Endonuclease- 1-Reduction/oxidation Effector Factor 1 (APE1/Ref-1) is a multi-functional protein with endonuclease activity in DNA base excision repair and redox signaling activity. This redox activity is responsible for reducing oxidized cysteines on specific transcription factors, including hypoxia inducible factor 1 alpha (HIF1a), enabling them to bind target sequences in DNA. We evaluated the mechanisms underlying PDAC cell responses to hypoxia and APE1/Ref-1 redox signaling control of HIF1a, a critical factor in hypoxia- induced CA9 transcription. We hypothesized that obstructing the HIF-CA9 axis at two points via APE1/Ref-1 inhibition (which results in a decrease in CA9 expression) and direct CA9 inhibition results in enhanced PDAC cell killing under hypoxic conditions.

In our studies, HIF1a-mediated induction of CA9 is significantly attenuated following APE1/Ref-1 knock down or redox signaling inhibition in patient-derived PDAC cells and pancreatic cancer-associated fibroblast cells using the APE1/Ref-1 redox signaling inhibitor APX3330 (currently in clinical trials). Additionally, dual-targeting of APE1/Ref-1 redox signaling activity and CA9 activity results in additive-to-synergistic enhancement of acidification and cytotoxicity of PDAC cells under hypoxic conditions as well as decreased tumor growth in an ex vivo 3-dimensional tumor co-culture model. These studies are clinically relevant as we used the CA9 inhibitor SLC-0111 (phase I clinical trial completed), as well as APX3330 (IND 125360), for which a phase I clinical trial has opened.

Further experiments characterized novel analogs of APX3330: APX2009 and APX2014, which demonstrated up to 50-fold improved potency as measured by pH reduction, cytotoxicity, and inhibition of hypoxia-induced CA9 expression. An SLC-0111 analog, FC12-531A, demonstrated up to 75-fold improved potency as measured by cytotoxicity. In combination, these analogs resulted in synergistic inhibition of 3D tumor spheroid growth at nanomolar-to-low-micromolar concentrations. These results underscore the concept that proper combination therapy has significant clinical utility of blocking APE1/Ref-1 and CA9 function for novel PDAC therapeutic treatment.

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

POSTER #122

FINAL RESULTS OF PHASE I/II STUDY OF COMBINATION OF SORAFENIB, VORINOSTAT, AND BORTEZOMIB IN ACUTE MYELOID LEUKEMIA WITH FLT3-ITD MUTATION OR POOR-RISK CYTOGENETICS

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Introduction

We report the final results of a phase I/II study of combination of targeted agents sorafenib, vorinostat, and bortezomib (SorVorBor) in poor-risk AML. Data from the phase I part of this study have previously been reported by our group. The rationale for SorVorBor phase I/II study was based on findings of an initial phase I study, at Indiana University, of the combination of sorafenib and vorinostat (SorVor) in patients with AML. The SorVor study suggested a potential benefit in terms of response to treatment in two patient groups: (i) patients with *FLT3-ITD* mutation, and (ii) patients with poor-risk cytogenetics (monosomy 5 or 7, or complex profile). The SorVor study also suggested a potential synergistic action offered by inhibition of *p52NFKB*, a downstream target of proteasome inhibition. These findings laid the hypothetical ground for the addition of bortezomib to SorVor, aiming at achieving synergism by proteasome inhibition, in the SorVorBor study (NCT01534260).

Methods

Adult patients with an ECOG performance status 0-2, adequate kidney and liver function, and a diagnosis of relapsed/refractory AML or those age 60 or older with untreated disease were enrolled. Patients had to have *FLT3-ITD* mutation and/or poor-risk cytogenetics (monosomy 5, monosomy 7 or complex cytogenetics). Phase I consisted of cohorts with escalating doses of sorafenib, vorinostat, and bortezomib. An MTD was not achieved in the phase I part of this study, therefore the safe dosing determined for the phase II part was: sorafenib 400 mg BID, vorinostat 200 mg BID (both for 14 days), and bortezomib 1.3 mg/m^2 IV on days 1, 4, 8, and 11, every 21 days. Response was evaluated based on the revised guidelines by the International Working Group for AML.

Results

Seventeen patients were enrolled in the phase I part and 20 in the phase II. Three patients in cohort 5 of the phase I part received a similar dosing to patients in the phase II; therefore, the data from this cohort were added to the phase II data for response analysis. A total of 23 patients were consequently considered for analysis having received the optimal dosing previously delineated. Fourteen patients were evaluable for response after completing at least one cycle of therapy. Rest of the patients either did not complete the first cycle or did not have a bone marrow examination performed for variety of reasons. Evaluable patients received between one to three cycles of treatment. The median age was 58 years (24-76). One patient (7.1%) had previously untreated disease while 13 patients (92.9%) had relapsed and/or refractory disease. Seven patients (50.0%) had received three or more lines of therapy. One patient (7.1%) had relapsed following hematopoietic stem cell transplantation. Fifty percent of patients had *FLT3-ITD* mutation and 57.1% carried poor-risk cytogenetics. The most common toxicities were gastrointestinal disturbances (64.2%) including diarrhea, nausea, or vomiting reaching grade 2 in 14.2% of patients. No grade 3 or 4 gastrointestinal toxicities were observed. Other toxicities included fatigue (31.3%), rash (21.4%), and neutropenic fever (7.1%); none was greater than grade 2. Response was observed in four out of the 14 patients (28.6%) including one patient (7.1%) achieving CR, one patient (7.1%) achieving CRi, and two patients (14.3%) achieving PR. All four patients who responded had *FLT3-ITD* mutation. Eight out of the 10 non-responders (80.0%) and none of the four responders had poor-risk cytogenetics. All four responders were previously treated and had relapsed and/or refractory disease. The characteristics of the participants and their response are summarized in Table 1. Comparing results of the SorVorBor study to those from SorVor study suggests a lower overall response rate in the SorVorBor study (28.6% versus 53.8%).

Conclusion

The SorVorBor combination was safe and tolerable in patients with relapsed/refractory AML. There was no dose limiting toxicity per the phase I part. A clinical response was observed in 60% of patients with *FLT3-ITD* mutation and none with poor-risk cytogenetics. In SorVor stufy, all FLT3ITD+ responders achieved PR, while addition of bortezomib in this study resulted in 50% CR/CRi in responders with FLT3-ITD+ disease.

Translational/Clinical Research Resident

POSTER #123

PREDICTORS OF NODAL AND METASTATIC FAILURE IN EARLY STAGE NON-SMALL CELL LUNG CANCER AFTER STEREOTACTIC BODY RADIATION THERAPY

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Purpose/Objective(s): Up to 30% of early stage non-small cell lung cancer (NSCLC) patients undergoing stereotactic body radiation therapy (SBRT) develop metastatic disease. While lung parenchymal failures after lung SBRT are thought to be salvageable if detected early, development of nodal and distant metastases are challenging situations that can seriously compromise patient outcomes. We sought to identify factors predictive of metastasis after lung SBRT.

Materials/Methods: Overall, 363 patients with early-stage NSCLC that received SBRT in 3-5 fractions were included in this retrospective analysis. Multiple patient and tumor factors were analyzed for their association with time to a combined nodal and/or distant metastatic failure: sex; age; lobe involved; previous history of NSCLC; gross tumor volume (GTV); T stage; histology (adenocarcinoma, squamous cell carcinoma, NSCLC not otherwise specified, or no pathology); radiation dose (biologically equivalent dose using alpha/beta=10 [BED_{10}]); prescription dose; minimum, maximum, and mean dose to GTV; and parenchymal lung sites of failure (in-field, same lobe but not in-field, different ispilateral lobe, and contralateral lung). A metastasis risk score linear model using beta coefficients from a multivariate Cox model as weighting factors was built for variables significant on multivariate analysis. Median follow-up of the cohort was 5.8 years.

Results: GTV and radiation dose were significantly associated with metastases on univariate analysis. A previous history of NSCLC ($p=0.84$), histology ($p=0.45$), T stage ($p=0.49$), centrality ($p=0.55$), and lung parenchymal failures ($p>0.11$) were not associated with the development of metastasis. On multivariate Cox proportional hazards modeling, GTV and radiation dose remained significantly associated with time to metastasis ($p<0.001$ and $p=0.044$, respectively). A metastasis risk score model using GTV and radiation dose was built: [Risk score = (0.01611 x GTV) – (0.00525 x prescription dose (BED_{10}))]. Two risk score cutoffs separating the cohort into low, medium, and high risk were examined. Consistent with its design, the risk score identified significant differences in time to metastases between 61 low risk, 264 medium risk, and 38 high risk patients ($p<0.001$) with 2-year metastases-free estimates of 86.7%, 81.3%, and 48.8%, respectively, and 3-year estimates of 81.1%, 63.8%, and 38%.

Conclusion: GTV and radiation dose are associated with time to metastasis. A risk score grouping can be used to identify patients at low, medium, or high risk for metastasis after SBRT for early stage NSCLC.

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

POSTER #124

HISTOLOGY, TUMOR VOLUME, AND RADIATION DOSE PREDICT OUTCOMES IN NON-SMALL CELL LUNG CANCER PATIENTS AFTER STEREOTACTIC ABLATIVE RADIOTHERAPY

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Purpose/Objective(s): Histology, tumor volume, and radiation dose have been separately reported as possible predictors of recurrence in non-small cell lung cancer following stereotactic ablative body radiotherapy (SABR). However, it remains unclear if histology should be independently considered when choosing SABR dose prescriptions.

Materials/Methods: The study population included 508 patients with 561 lesions treated between 2000 and 2016, of which 442 patients with 482 lesions had complete dosimetric information. The primary endpoint was in-field tumor control censored by either death or progression. Involved lobe control was also assessed.

Results: At a median follow-up of 6.7 years, 3-year in-field control, involved lobe control, overall survival, and progression-free survival were 88.1%, 80.0%, 49.4%, and 37.2%, respectively. Gross tumor volume (GTV, HR=1.01 per mL, p=0.0044) and histology (p=0.0225) were independently associated with involved lobe failure; GTV (HR=1.013, p=0.001) and radiation dose to the GTV (cutoff of 110Gy, biologically effective dose with a/ß=10 [BED_{10}], HR=2.380, p=0.0084) were independently associated with in-field failure. For squamous cell carcinomas, lower prescription doses were associated with worse in-field control independent of GTV (= vs. >110Gy BED_{10}: HR=3.621, p=0.0147; 12Gyx4 or 10Gyx5 vs. 18Gy or 20Gyx3: HR=3.530, p=0.0447). For adenocarcinomas, there were no differences in in-field control observed using the above dose groupings (p=0.12 and p=0.31).

Conclusion: GTV, dose to GTV, and histology are important factors contributing to involved lobe and in-field recurrence risk. A dose local control relationship was observed for squamous cell carcinomas treated with commonly used SABR dose prescriptions. In the absence of level I data to guide management, we posit that GTV and histology should be considered to personalize radiation dose for SABR. We suggest lower prescription doses (i.e., 12Gyx4 or 10Gx5) should be avoided for squamous cell carcinomas if there are no concerns about normal tissue toxicity.

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

POSTER #125

TISSUE MICROARRAY ARTIFACTS AFFECTING QUANTITATIVE IMAGE ANALYSIS WITH ER, PR, AND HER2/NEU BREAST CANCER IMMUNOHISTOCHEMISTRY

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Tissue microarrays (TMAs) are vital to the field of pathology and are widely used for clinical cancer research. Multiple core biopsies are obtained from numerous paraffin-embedded blocks and are reassembled into a single block. Often TMAs are composed of an average of 50 to 200 individual cores (cases) on a single slide. In addition, the breast cancer cases studied contained over 90% of tumors which were invasive ductal carcinomas.

This study investigated artifacts and defects associated with cores in TMAs that occur and affect image analysis. To understand how these artifacts and defects affect the image analysis software, fourteen breast TMAs were analyzed with immunostains for ER, PR, and HER2/neu using the Aperio positive pixel algorithm. Some tissue microarrays contained natural artifacts, tissue preparation defects, and TMA building artifacts that falsely altered positive pixel readings.

Natural artifacts included: necrosis, hemorrhage, hemosiderin, melanin pigment, anthracosis, calcification, and surgical cauterization. Tissue features that caused issues included adipose tissue, stromal tissue, and inflammation. Tissue preparation defects included: precipitation of DAB staining, edge effect of the core staining, hematoxylin overstaining, and excessive glue on the slides, blush false staining, enzymatic digestion, missing cores, and minute glass particles. In addition, TMA technical processing errors during microtoming included: tears, partial cores, folds, and rims.

The presence of natural artifacts, tissue preparation defects, and TMA technical processing errors were found to misrepresent the true positive pixel percentages in the analyzed cores.

Examination of the fourteen TMAs with immunostains of ER, PR, and HER2/neu revealed defects in 20%, 23%, and 35% of cores respectively, taking out controls and intentional blanks. The presence of these alterations did artificially effect the positive pixel readings. These defective cores typically cause higher reported positive pixel values in the imaging data. In large studies such as this involving approximately 1,200 cases, not removing these aberrant cores can alter outcome of the study. This study showed ER, PR, and HER2/neu positive pixel staining of 54.33%, 38.07%, and 20.65% respectively which is consistent with previous studies. In conclusion, it is recommended that in TMA analysis, each slide be examined for these natural artifacts, tissue preparation defects, and technical processing issues and removed from the study to ensure accurate reporting of the data.

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

POSTER # 126

ROLE OF TET2 IN MAMMARY STEM CELL FATE DECISION

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Epigenetic mechanisms, including DNA methylation, plays an important role in regulation of stem cell fate and tumorigenesis. The Ten-Eleven-Translocation (TET) 2 is a core enzyme for DNA demethylation by catalyzing the conversion of 5-methylcytosine (5mC) to 5-hydormethylcytosine (5hmC). It has been shown that TET2 is a main regulator of hematopoietic stem cell homeostasis and loss of TET2 closely associated with hematopoietic malignancies. Interestingly, our recent study has revealed that TET2 directs the breast cancer stem cell like population cell fate to the differentiated cells in vitro. Since aberrant stem cell fate is often promotes tumorigenesis, defining the role of a key epigenetic regulator, such as TET2, which governs the mammary stem cell (MaSC) fate will provide the novel mechanism of MaSC fate decision as well as new strategies for breast cancer treatment. Thus, mammary gland specific *Tet2* knock-out mouse model (MMTV-Cre;*Tet2* $^{fl/fl}$) is used to determine the physiological and pathological roles of TET2 in the regulation of MaSC fate and mammary gland development. Our data show that deletion of *Tet2* in mouse mammary gland leads to disrupted mammary epithelial morphology, defective lobular alveolar development, along with aberrant mammary stem cell fate decision, contributing to an expansion of the MaSC population and abnormal development of mammary gland. In conclusion, the data demonstrates that TET2 plays an important role in controlling MaSC fate to maintain the mammary epithelium homeostasis, and loss of TET2 may promote mammary malignancies.

Basic Science ***Graduate Student***

POSTER # 127

PROTEOMIC ANALYSIS IDENTIFIES A LIPPIA ORIGANOIDES EXTRACT TARGETS MITOCHONDRIAL METABOLISM IN TRIPLE-NEGATIVE BREAST CANCER.

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Triple-negative breast cancer (TNBC) is an aggressive subtype with low 5-year survival rates, high 3-year recurrence rates, and no known therapeutic targets. Recent studies indicated TNBCs possess an altered metabolic state with higher rates of glycolysis, mitochondrial oxidative phosphorylation (OXPHOS), and increased generation and utilization of tricarboxylic acid (TCA) cycle intermediates. We utilized label-free quantitative proteomics to gain insight into the anti-cancer mechanisms of a methanolic extract from the Central American plant *Lippia origanoides* (L42) on MDA-MB-231 TNBC cells 1,2. L42 treatment dysregulated mitochondrial OXPHOS by targeting Complex I of the electron transport chain and suppressed cellular metabolism by targeting key TCA cycle enzymes and mitochondrial lipid and amino acid metabolic pathways 3. Overall, our results reveal new compelling evidence that L42 triggers rapid irreversible apoptosis in MDA-MB-231 cells by effectively 'starving' the cells of metabolites and ATP. We continue to study the specific bioactive components of the extract in the search for novel, highly effective mitochondrial inhibitors to selectively target TNBCs.

Basic Science ***Graduate Student***

POSTER # 128

NRP1:FGFR1 SIGNALING COMPLEX: A NEW APPROACH TO TREAT ERBBI-RESISTANT BREAST CANCERS

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ABSTRACT

Background: Human epidermal growth factor receptor 2 (ErbB2)-amplified Breast cancers (BC) initially response to treatment with ErbB2-targeted antibodies and kinase inhibitors (collectively called as ErbB inhibition or ErbBi) at a high rate, but ultimately develop an acquired resistance and metastatic recurrence. Thus, there is a critical need to uncover metastatic events that drive the failure of ErbBi therapy. We have recently performed a global gene expression analysis of several ErbBi resistant cell lines. The preliminary data demonstrate enhanced expression of fibroblast growth factor receptor (FGFR1) and the axonal guidance molecules known as neuropilins (Nrp) during the EMT event that is brought about upon acquisition of resistance to ErbBi. This gene expression analysis together with mass spectroscopy data strongly suggest that upon induction of EMT, FGFR1 forms a physical complex with β3 integrin and Nrp1. We therefore seek to address the hypothesis that EMT-driven ErbBi resistance is facilitated by an Nrp1:FGFR1 matrixsensing signaling complex. We also aim to determine if genetic ablation or pharmacological disruption of this complex can overcome metastatic resistance to ErbBi.

Methods and Results: Nrp1- blocking antibodies from Genetech and Nrp1-ShRNA were used to deplete Nrp1 in D2A1 murine mammary tumor cell lines. Nrp1 and FGFR1 were also overexpressed and depleted in Normal murine mammary gland cells (NMuMG) cells. All these cells were evaluated for downstream cell signaling, FGFR1:Nrp1: Integrin interactome and growth in 2D and 3D culture. Some of the key findings from our study are: 1)The NRP1 protein expression is elevated in resistant cell lines as compared to their non-resistant counterparts 2) Co-immunoprecipitation assays shows that FGFR1 and Nrp1 form a physical complex that also contains b3 integrin and formation of this ternary complex is dependent on the expression of Nrp1. 3) Depleting or inhibiting NRP1 destabilizes FGFR1 basal levels. 4) Nrp1-depleted cells showed decrease in growth in 2D and 3D culture and 5) Combination of FGFR1 inhibitor and Nrp1 depletion has enhanced anti-growth effect on D2A1 cells. In conclusion, our preliminary data identify Nrp1 and FGFR1 complex novel mediators of matrix-associated ErbBi resistance.

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

POSTER # 129

GFI1-SPHK1 AXIS MAINTAINS GROWTH AND SURVIVAL OF MYELOMA CELLS

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Multiple myeloma (MM) is an incurable hematologic malignancy caused by the accumulation of malignant plasma cells in the bone marrow featuring osteolytic lesions in the majority of patients. We previously reported that Growth independent factor 1 (Gfi1) upregulation in bone marrow stromal cells (BMSC) causes suppression of osteoblast differentiation. Our recent findings show that Gfi1 is also increased in the majority of $CD138^+$ cells from MM patients and cell lines. We hypothesizes that adhesive interactions between MM cells and BMSC stimulate survival and growth of MM cells in part though Gfi1-SphK1 axis by modulating their sphingolipid profile (Cer/SPH/S1P ratio).

The effects of Gfi1 knock down (KD) on MM cell survival were assessed by transduction with pLKO.1-puro lentivirus encoding Gfi1 or non-mammalian shRNA. The anti-apoptotic effects of Gfi1 over-expression (o/e) were tested by transduction with pUC2 lentivirus encoding Gfi1 or the empty vector (EV).

We found that SphK1 mRNA is highly expressed in $CD138^+$ cells from MM patients and cell lines compared with normal donors and that Gfi1 protein levels correlate with the expression of active SphK1. Microenvironmental soluble factors (IL-6 and S1P), hypoxia and adhesive interactions with BMSC further increased Gfi1 and SphK1 mRNA and protein levels in MM cells. KD of Gfi1 induced a profound decrease of SphK1 mRNA levels and protein activity in MM cells that inhibited their growth and viability. Gfi1 o/e had opposite effects on SphK1 levels and conferred a survival advantage to MM cells over control cells. This was mirrored by increased intracellular S1P and decreased sphingosine levels as measured by LC-MS/MS. A SphK1 specific inhibitor (SKI2) profoundly reduced cell viability in MM cells regardless of p53 status. In p53 replete MM cells, SphK1 inhibition significantly reduced c-Myc protein expression, induced autophagy as shown by increased LC3 II protein levels and increased total ceramides levels. Moreover, in a 3D model, BMSC protected p53 replete MM cells from the anti-survival effects of SKI2. These data suggest that Gfi1 regulates MM growth in part via enhancing the expression and the activity of SphK1.

Taken together, our results support that Gfi1 acts as a key regulator of MM growth and survival at least partially through modulation of SphK1. Therefore, targeting Gfi1 may be a novel therapeutic strategy for MM patients.

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