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POSTER #1

CHEMOTHERAPY AND CANCER-INDUCED CACHEXIA YIELD DISTINCT METABOLIC PERTURBATIONS

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Advanced cancer patients frequently suffer from cachexia, a severe wasting condition characterized by depletion of both muscle and adipose tissue. We recently showed that chemotherapy, one of the primary treatment options for cancer, can also lead to cachexia. In this study we conducted a metabolomics investigation on murine models of cancer and chemotherapy to understand how each contributes to cachexia. The cancer group consisted of mice implanted with C26 colorectal tumor cells and the chemotherapy group were administered Folfiri (5-FU, leucovorin, irinotecan) for five weeks. Metabolomics analyses used an untargeted, NMR-based approach on serum and muscle tissue. Consistent with previous studies, serum from the C26 tumor group demonstrated a reduction in circulating branched chain amino acids (BCAA), possibly due to increased catabolism of those amino acids in the wasting muscle. Conversely, the BCAA levels in the Folfiri group were unchanged suggesting that this catabolic pathway does not contribute to chemotherapy-induced wasting. Interestingly, the levels of circulating acetate, a primary derivative of gut microbial metabolism, were greatly diminished in the C26 hosts, but unaffected in the Folfiri group, suggesting that the C26 cancer group may be uniquely dysbiotic.

Metabolomics analysis of muscle tissue in both C26 and Folfiri groups showed alterations in the levels of several amino acids including valine, glutamate, glycine and tyrosine. Distinct reductions in succinate in both groups suggest a decrease in oxidative metabolism, in line with our previous findings. Further, an increase in the endogenous antioxidant anserine in the Folfiri group suggests that oxidative stress may be at the root of this perturbation rather than reliance on another energetic pathway.

Our findings support the idea that the understanding of the unique features of chemotherapy-induced cachexia may help in the design of modified or supplemental treatments that can mitigate this severe adverse condition in cancer patients.

Basic Science ***Faculty***

POSTER #2

ROLE OF MEGAKARYOCYTE PHOSPHATIDYLINOSITOL TRANSFER PROTEINS ALPHA AND BETA IN TGF-BETA-MEDIATED REGULATION OF HEMATOPOIESIS

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Deciphering hematopoietic stem (HSC) and progenitor (HPC) cell regulation is important for better understanding and treating hematopoietic diseases. Megakaryocytes (Megs) have been linked to the microenvironmental regulation of HSCs and HPCs, but the signaling events within megakaryocytes regulating hematopoiesis remain unclear. An important signaling pathway in megakaryocytes and platelets is the phosphoinositide pathway. The phosphoinositide pathway contributes to events linked to the cytoskeleton and plays an important role in regulating platelet adhesion and plug formation pathways. Phosphatidylinositol is a rare membrane structure lipid, but is critical for cellular signaling upon phosphorylation by lipid kinases to generate phosphoinositide. Critical to this pathway are phosphatidylinositol transfer proteins (PITPs) that *in vitro* enhance transfer of aqueous insoluble phosphatidylinositol from one membrane to another. Class I PITP proteins PITPa and b are highly conserved, small, and ubiquitously expressed in mammalian cells. To test the hypothesis that phosphatidylinositol signaling contributes to hematopoiesis, we generated conditional knock out mice that lack either *pitp*a single isoform (*pitp*a$^{fl/fl}$*pf4*Cre^{+}) or both *pitp*a and *pitp*b (*pitp*a$^{fl/fl}$b$^{fl/fl}$*pf4*Cre^{+}) specifically in their platelets and megakaryocytes. Bone marrow (BM) from *pitpa* and *pitpa/b* KO mice manifested decreased numbers of phenotypically-defined HSCs (~3 fold decrease), megakaryocyte-erythroid progenitors (~1.8 fold decrease), and functional cycling HPCs (CFU-GM, BFU-E, CFU-GEMM) compared to littermate controls. Conditioned medium (CM) from thrombopoietin-cultured *pitpa* and *pitpa/b* KO BM-derived Megs, but not littermate control cells, suppressed colony formation of HPCs from normal C57Bl/6 BM. *Pitpa* and *pitpa/b* KO Meg CM contained higher levels of selected cytokines and chemokines than control Meg CM. This included transforming growth factor (TGF)-b and IL-4, both with known myelosuppressive activities. Myelosuppressive Meg CM from *pitpa* and *pitpa/b* KO BM was completely neutralized *in vitro* by monoclonal anti-TGF-b antibody and partially by anti-IL-4 antibody. IL-4 synergized with TGF-b*in vitro* as a suppressive agent. Importantly, treatment of *pitpa/b* KO mice with anti-TGF-b antibodies completely restored normal BM HSC and HPC numbers. Our studies thus link megakaryocyte phosphoinositide synthesis with homeostatic regulation of hematopoiesis through the controlled release of TGF-b and IL-4.

Basic Science ***Faculty***

POSTER #3

LMO2'S ONCOGENIC FUNCTION IN T-CELL LEUKEMIA REQUIRES LDB1

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LIM domain Only-2 (LMO2) is one of the most frequently deregulated oncogenes in T-cell acute lymphoblastic leukemia (T-ALL). *LMO2* encodes a small protein with 2 LIM domains that is part of a large multiprotein complex in hematopoietic stem and progenitor cells (HSPC), where it is required for HSC specification and maintenance. Many of LMO2's protein partners in HSPCs are expressed in T-ALL implying that protein complexes like those scaffolded by LMO2 in HSPCs also play a role in leukemia. In this study, we analyzed a critical component of the LMO2 associated complex, LIM domain binding 1 (LDB1). LDB1 appears to be an obligate partner of LMO2 in HSPCs but it is not required for T-cell development from committed progenitors. LDB1 is concordantly expressed with LMO2 in human T-ALL although its expression is more widespread than LMO2. To further define Ldb1's role in leukemia, we induced its conditional knockout in *CD2-Lmo2* transgenic mice. *CD2-Lmo2* transgenic mice develop T-ALL with high penetrance and closely model the human disease. We discovered that Lmo2-induced T-ALL was markedly attenuated in penetrance and latency by Ldb1 deletion. Since Lmo2 induces a distinct differentiation arrest in T-cell progenitors prior to leukemic transformation, we analyzed the differentiation of T-cell progenitors in CD2-Lmo2 transgenic/Ldb1^{flox}/Lck-Cre mice and in non-Lmo2 transgenics: Ldb1^{flox}/Lck-Cre mice. Ldb1 deletion by Lck-Cre was efficient in double negative and double positive T-cell progenitors. In striking contrast, Ldb1 deletion could not be effected in CD2-Lmo2 transgenic T-cell progenitors. Consistent with this finding, T-ALLs that developed in CD2-Lmo2/floxed-Ldb1/Lck-Cre mice had incomplete deletion of Ldb1. These results imply that Ldb1 is a required factor for Lmo2 to induce T-ALL. Lastly, gene expression analysis of Lmo2-induced T-ALLs and ChIP-exonuclease analysis of Ldb1 occupancy in T-ALL suggested that the Lmo2/Ldb1 complex enforce a gene signature like that seen in HSPCs and in Early T-cell Precursor ALL. In conclusion, Ldb1 is a required partner for Lmo2 to induce T-ALL. Additionally, the HSPC function of Lmo2/Ldb1 complexes may be recapitulated in T-cell progenitors prior to T-ALL.

Basic Science ***Faculty***

POSTER #4

DEVELOPMENT OF SMALL MOLECULE INHIBITORS FOR CANCER THERAPY BY TARGETING RPA AND XPA NUCLEOTIDE EXCISION REPAIR (NER) PROTEINS

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Targeting DNA repair and the DNA damage response 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 these patients, and 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. To this end, we have developed a series of novel small chemical molecules that disrupt critical protein-DNA interactions in the nucleotide excision repair (NER) pathways. It is well understood that various cancer treatments like cisplatin, etoposide and ionizing radiation 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 nucleotide excision repair (NER) or homologous recombination repair (HRR) reduces the effectiveness of chemo- or radio- therapy. Replication protein A (RPA) and Xeroderma Pigmentosum Group A (XPA) plays a crucial role in the NER pathway and makes them a novel drug target to develop novel cancer therapy. We anticipate both direct mechanisms of action on the repair pathways and synthetic lethal interactions can be exploited for therapeutic benefit. The series of novel small molecule inhibitors that we have developed targeting RPA and XPA proteins independently exhibit single-agent anti-cancer activity in cancer cell lines, and potentiate cellular sensitivity to chemotherapeutic agent. Data demonstrate that these novel inhibitors do not interact with DNA but directly bind the corresponding NER proteins. Our data demonstrate that this class of inhibitors can be further developed as an anti-cancer therapeutic with considerable potential to be used in conjunction with radiation therapy and other cancer therapies that induce DNA damage.

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Basic Science ***Faculty***

POSTER #5

EXTINGUISHING IMMUNOSUPPRESSIVE MYELOID CELLS TO ENHANCE CANCER IMMUNOTHERAPY

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Introduction: Prostate cancer (PCa) is the most common noncutaneous malignancy in men in the United States. A significant fraction of advanced PCa treated with androgen deprivation therapy experience relentless progression to lethal metastatic castration-resistant prostate cancer (mCRPC). The PCa tumor microenvironment is comprised of a complex mixture of epithelial and stroma cell types engaged in multifaceted heterotypic interactions functioning to maintain tumor growth and immune evasion. We recently uncovered the important role played by myeloid-derived suppressor cells (MDSCs) to mediate tumor immune evasion in aggressive PCa (Wang*, Lu* et al. *Cancer Discovery*, 2016). Immune checkpoint blockade (ICB) has elicited durable therapeutic responses across a number of cancer types. However, the impact of ICB on mCRPC has been disappointing, which may signal the need to combine mechanistically-distinct ICB agents and/or override immunosuppression in the tumor microenvironment. Our objective is to determine if robust immunotherapy responses in mCRPC may be elicited by the combined actions of ICB agents together with targeted agents that neutralize MDSCs yet preserve T cell function.

Methods: We created a novel embryonic stem cell (ESC)-based chimeric mouse model of mCRPC engineered with signature mutations to study the response to single and combination immunotherapy. The efficacy studies were followed with detailed mechanistic investigation and clinical validation.

Results: Consonant with early stage clinical trials experience, anti-CTLA4 or anti-PD1 monotherapy failed to impact disease progression. Similarly, modest anti-tumor activity was observed with combination ICB as well as monotherapy with targeted agents including Cabozantinib (tyrosine kinase inhibitor), BEZ235 (PI3K/mTOR inhibitor), and Dasatinib (tyrosine kinase inhibitor). In contrast, mCRPC primary and metastatic disease showed robust responses to dual ICB treatment together with either Cabozantinib or BEZ235, but not with Dasatinib which impaired T cell infiltration in the tumor. Detailed intratumoral immune profiling with mass cytometry (CyTOF) showed that combined ICB and Cabozantinib or BEZ235 was associated with significant depletion of MDSCs. Cabozantinib and BEZ235 blocked the PI3K signaling activity in MDSCs and reduced their immunosupppresive activity. Mechanistically, the combination efficacy was due to the upregulation of IL-1RA and suppression of MDSC-promoting cytokines secreted by mCRPC cells.

Conclusions: We demonstrated that an antibody cocktail targeting CTLA4 and PD1 was insufficient to generate effective anti-tumor response, but combination of ICB with targeted therapy that inactivates PI3K signaling displayed superior synergistic efficacy through impairing MDSCs in the tumor microenvironment. These observations illuminate a clinical path hypothesis for combining ICB with MDSC-targeted therapies in the treatment of mCRPC (Lu et al. *Nature*, in press). Importantly, conclusions from the study on prostate cancer may have implications in combination immunotherapy for aggressive breast cancer which is also largely resistant to immune checkpoint blockade and replete with immunosuppressive myeloid cells.

Basic Science ***Faculty***

POSTER #6

PI3KINASE INACTIVATION THROUGH COMBINED LOSS OF CATALYTIC SUBUNITS P110A, P110ß AND P110D DERAILS HEMATOPOIETIC HOMEOSTASIS

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PI3Kinase is a lipid kinase that is involved in diverse cellular processes including cell growth, survival, differentiation and cell cycle. Deregulation of PI3K pathway has been associated with wide variety of cancers including several hematological disorders such as acute myeloid leukemia (AML). Hematopoietic growth factors such as Fms Related Tyrosine Kinase 3 (FLT3) ligand, stem cell factor (SCF), erythropoietin (EPO), and thrombopoietin (TPO) activate PI3K signaling pathway via tyrosine kinase receptors, growth factor receptors and G-protein coupled receptors. In mouse models of abrogation of PI3K signaling, involving AKT deletion, impairs the self-renewal potential of hematopoietic stem cells (HSCs). In contrast, mice models with constitutive activation of PI3K signaling, involving deletion of PTEN phosphatase, causes increased cycling of HSCs. PI3K regulatory subunits, P85a and P85ß, are critical for fetal liver hematopoiesis. However, the role of PI3K in adult hematopoiesis and contribution of catalytic subunits of PI3K to hematopoietic homeostasis is not well studied. Given that HSCs express all three catalytic subunits of PI3K, we developed a mouse model for ablated PI3K pathway by depleting PI3K catalytic subunits p110a, p110ß and p110d. p110a and p110ß subunits were conditionally deleted via MX-Cre system upon Poly-IC injection, and p110d inactivation was achieved through p110d inactivating knock-in mutation. Mouse tissues were harvested upon 5 weeks post poly-IC, at which time point the mice appeared moribund and were therefore analyzed for various hematopoietic lineages. These mice were anemic and peripheral blood analysis showed leukopenia, neutropenia and anemia with significantly reduced WBC, neutrophils, RBC, hemoglobin, hematocrits and platelet counts. Mice depleted of p110a, ß and d subunits-p110 triple knock out mice (p110-TKO) – demonstrated significantly reduced spleen size, reduced bone marrow cellularity with anemic bones relative to wild type mice. Hematopoietic stem cell compartment in p110-TKO mice showed increased frequency in Lineage^{-ve}Sca1^{+ve}Kit^{+ve} (LSK) population as well as an increase in more primitive HSCs defined as SLAM cells (CD150^{+ve}CD48^{-ve}). In contrast, there was reduction in total number of common lymphoid progenitors (CLP), common myeloid progenitors (CMP), granulocyte-macrophage progenitors (GMP) and megakaryocyte-erythroid progenitors (MEP) in the bone marrow. These mice also showed dramatically reduced total B-cells and significantly reduced T-cells. However, these mice show increased frequency of GR1-Mac1 positive granulocytes in all hematopoietic compartments. Erythroid compartment was analyzed for various stages of red blood cell development using Ter119 and CD71 markers. Hampered RBC development was observed in p110-TKO mice with significantly increased immature erythroid cells (Ter119^{low}CD71^{low}) in the bone marrow, spleen and peripheral blood compartments, with significantly reduced terminally differentiated cells (Ter119^{+ve} only). HSC self-renewal studies involving competitive repopulation assay showed that recipient mice have significantly reduced p110-TKO donor chimerism. Earlier studies from others have reported that p110a and p110d subunits are dispensable for adult HSC function, however p110a is required for terminal erythroblast maturation and p110d is required for leukocyte development. Our studies revealed that lack of all three catalytic subunits of PI3Kinase significantly impairs hematopoietic homeostasis with hampered stem cell development, erythroid cell development and a complete shut down in B-cell production.

Basic Science ***Faculty***

POSTER #7

HISTONE DEACETYLASE 6 (HDAC6) INHIBITORS RETARD CELL PROLIFERATION AND INDUCE ROBUST CELL DEATH IN GLIOBLASTOMA (GBM) CELLS AND GBM SPHEROIDS CONTAINING STEM CELLS

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Glioblastoma (GBM) comprises the most common and very aggressive form of primary brain tumor with a dismal prognosis and very poor response to the current therapies. HDAC inhibitors (HDACi) induce growth arrest and apoptosis in tumor cells. HDAC6 is a unique enzyme having two deacetylase domains, and an ubiquitin-binding domain. HDAC6 interacts with a number of proteins in the cytoplasm and is involved in tumorigenesis, cell motility, and metastasis. In this study, we evaluated HDAC6 as a relevant target for GBM treatment by using potent HDAC6 inhibitors, CAY 10603, ACY-1215, and Tubastatin A. The ACY-1215 and Tubastatin and HDAC6 siRNA knockdown. All inhibitors were selected due to favorable blood-brain-barrier permeability characteristics. Our data indicate that HDAC6 inhibitors target the established GBM cell line U87MG (U87), the early passage GBM10 and M-HBT-161 cultures, and CD133- and SOX2-positive stem cell-like spheroids. The inhibitors at 1-8 μM triggered significant inhibition of cell survival and induced apoptotic cell death in GBM cells and GBM spheroids. CAY 10603, Tubastatin A, and ACY-1215 reduced cell survival in U87MG cells by 50% (IC50) at 1.07, 5.83, and 6.27 μM treatment for 96 h, respectively. Moreover, CAY 10603-triggered cell death in U87MG cells as well as CD133/SOX2-expressing spheroids and was associated with activation of caspases-3, -6, and -9 and the general caspase-inhibitor, Z-VAD-FMK significantly inhibited CAY 10603-triggered cell death at 10 μM treatment for 48 h. Overall, our results show that these HDAC6 inhibitors robustly inhibit the growth of GBM cells, and are effective in eliminating spheroids that contain GBM cancer stem cells which play a major role in drug resistance and disease recurrence. These results suggest that using HDAC6 inhibitors alone or in combination with other agents may potentially improve the survival of brain tumor patients.

Basic Science ***Faculty***

POSTER #8

SYSTEMIC ACTIVIN RESPONSE TO PANCREATIC TUMOR: IMPLICATION FOR EFFECTIVE CANCER CACHEXIA THERAPY

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Cancer cachexia decreases quality of life and increases mortality. Majority of patients with pancreatic ductal adenocarcinoma (PDAC) develop cachexia. Serum activin correlates with cachexia and reduced survival in PDAC and activin can cause cachexia without tumor involvement. We sought to characterize activin expression, identify mechanisms of expression, and test the potential utility of targeting activin for cachexia. We developed LSL-**K**rasG12D;LSL-**p**53R172H;Pdx1-**C**re (KPC) mouse models of orthotopic PDAC cachexia and observed that KPC mouse-derived cell lines expressing more activin caused more severe cachexia. Immunohistochemistry (IHC) detected activins not only in tumor but also in organs of tumor-bearing mice. RT-qPCR identified tumor-derived activin as INHBA while organs expressed both INHBA and INHBB. Query of the Oncomine and the Cancer Genome Atlas (TCGA) pancreatic cancer databases indicate that *INHBA*, but not the *INHBB*, is increased in human PDAC tumors and correlates with reduced survival of PDAC patients. In samples from a cohort of PDAC patients from IU Hospital, IHC detected activins in tumor cells and the surrounding stroma, and in muscles as well. Tumor-secreted factors might mediate the organ response because KPC conditioned medium induced activin in cultured mouse muscle cells and myotube atrophy. Additionally, soluble activin receptor ACVR2B/Fc had a limited inhibitory role in higher activin-expressing PDAC cachexia and it did not affect activin expression in organs. Overall our results indicate that PDAC induces a systemic activin response by secreting still unidentified factors and targeting both the tumor-released and tumor-induced activins would offer more effective therapeutic options.

Basic Science ***Faculty***

POSTER #9

TEMPORAL REGULATION OF HYDROGEL STIFFNESS THROUGH ORTHOGONAL ENZYMATIC REACTIONS

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Introduction: Hydrogels with temporally controlled mechanical and biochemical properties are highly useful for studying important cancer cell fate processes.In particular, two-step crosslinking strategies have been employed to 'stiffen' cell-laden hydrogels. Often, these stiffening strategies involve UV-light and radical induced polymerization, which may not be ideal for cell-based applications. Here, we aimed at exploiting orthogonal, enzymatic reactions, namely Sortase A (SrtA) and mushroom tyrosinase (MT), to synthesize and tune the mechanical properties of synthetic hydrogels. SrtA is a transpeptidasethatcovalently ligates two peptide sequences, G_n and LPXTG where X is any amino acid. On the other hand, MT oxidizes tyrosine residues into di-tyrosine. Due to the aforementioned advantages, we developed PEG-peptide hydrogels that can be crosslinked by SrtA-mediated transpeptidation and subsequently stiffened through MT-mediated di-tyrosine formation.

Methods: Macromer 8-arm poly(ethylene glycol) norbornene (PEG8NB) and photoinitiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) were synthesized per established protocols**.** All peptides (i.e, GGGGYC, CYLPRTG) were synthesized by microwave-assisted solid phase peptide synthesis. PEG-peptide conjugates were prepared through reacting purified peptides with PEG8NB via thiol-norbornene photoclick chemistry in the presence of LAP (5 mM) and light (365 nm, 40 mW/cm^2). Hepta-mutant his-tagged Srt A was expressed in BL21 *E. Coli* and was purified by Ni^+ Column chromatography.Hydrogels were fabricated with SrtA and a 1:1 stoichiometric ratio of 8-arm PEG-LPRTG and GGGGY-PEG. Gelation kinetics at varying SrtA concentrations were monitored via *in situ* oscillatory rheometry. SrtA-crosslinked gels were incubated in PBS for 24 hours before measuring elastic modulus (G'). Temporal control of the hydrogel mechanical properties was achieved through incubating the gels with MT (1 kU/mL) for 6 hours at 37^{o}C.

Results: The gelation kinetics of PEG-peptide hydrogels was investigated by in situ rheometry using 3wt% PEG-peptide conjugates and varying concentrations of SrtA (i.e., 150 μM, 300 μM, 600 μM).The gelation was accelerated at higher SrtA concentrations as indicated by rapid increases in G'. Formulations containing 2, 3, and 4 wt% total PEG concentration yielded gels with initial elastic moduli of ~3000, ~6000, and ~10000 Pa, respectively. Temporal stiffening was achieved by fabricating 3 wt% hydrogels of PEG-LPRTG and GGGGY-PEG followed by incubation in MT solution for 6 hours. The addition of MT to the gels increased the gel modulus roughly two-fold, demonstrating the feasibility of using orthogonal enzymatic reactions for gel crosslinking and in situ stiffening.

Conclusions: Here, we have established an enzymatic gelation scheme with tunable initial crosslinking and temporal regulation of gel stiffness. The use of sequential enzymatic reactions allows for dynamic tuning of primary and secondary crosslinking without the necessity of co-initiators and UV-light. In addition, SrtA and MT reactions yield no cytotoxic by-products, thereby making them advantageous, cytocompatible systems for studying cancer cell response to dynamic changes in the microenvironment.

Basic Science ***Graduate Student***

POSTER #10

THE ROLE OF WNT5A SIGNALING PATHWAY IN OVARIAN CANCER PROGRESSION

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Ovarian Cancer (OvCa) is the most fatal gynecological malignancy and the 5th leading cause of cancer death among U.S. women. The majority of women with OvCa (75%) have a very low survival rate (30%), as OvCa is usually diagnosed in late stages after development of intra-peritoneal metastasis. Thus, it is indispensable to understand the molecular mechanisms that contribute to OvCa metastatic success, in order to design effective treatment strategies to improve the overall survival of women with OvCa. Wnt5a is a non-canonical Wnt ligand that binds to several cell membrane receptors and activates many downstream signaling pathways that are fundamental for normal developmental processes during embryogenesis. In the past decade, the aberrant activation or inhibition of Wnt5a signaling is emerging as an important event in cancer progression, exerting both oncogenic and tumor suppressive effects. The role of Wnt5a in OvCa is controversial, as studies report conflicting data. In addition, mechanistic data regarding the contribution of Wnt5a to OvCa progression are largely unavailable. The main aim of this research is to obtain a molecular level understanding of Wnt5a signaling in OvCa and to investigate its potential roles in influencing OvCa metastatic success.

Our Data show Wnt5a is prevalent in ascites samples from women in different stages with OvCa, suggesting a role for Wnt5a in promoting disease progression. Data obtained from TCGA (n=583) show high expression of Wnt5a in primary ovarian tumors. Furthermore, Wnt5a enhanced OvCa cells adhesion, migration and invasion in a panel of organotypic and *ex vivo* functional assays. This was combined with striking morphological changes characteristic of an invasive phenotype in OvCa cells treated with recombinant Wnt5a protein and formation of tunneling nanotubes (TNT). Overall, our data suggests that Wnt5a plays an oncogenic role in epithelial ovarian cancer cells. More experiments exploring Wnt5a activated pathways and effects in epithelial ovarian cancer cells are underway.

Basic Science ***Graduate Student***

POSTER #11

SELECTIVE PHARMACOLOGICAL INHIBITION OF NOTCH RECEPTOR 3 SIGNALING INDUCES MYELOMA CELL DEATH AND PRESERVES OSTEOCYTE VIABILITY

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Multiple myeloma (MM) cell growth and survival are highly dependent on interactions between MM cells and cells in the bone/bone marrow microenvironment. Earlier work demonstrated that osteocytes (Ot), major regulators of osteoblast and osteoclast activity and the most numerous cells in bone, contribute to MM growth and the associated bone disease. In addition, MM/Ot interactions activate bidirectional Notch signaling between MM cells and Ot, which in turn enhances MM growth and increases Ot apoptosis. Further, these effects were mediated by Notch receptor (R)3 and R4 and prevented *in vitro* by pharmacological inhibition of Notch using a g-secretase inhibitor. Although systemic inhibition of Notch using g- secretase inhibitors has anti-MM activity, it causes significant toxicity *in vivo*, thus limiting its use in patients. The goal of this study was to assess *in vitro* the effects of selective inhibition of Notch R3 signaling on Notch activation and viability of MM cells and Ot. Towards this end, we used APX3330, a novel, oral, first-in-class human Ref-1 inhibitor that downregulates Notch R3 expression in solid tumors. In murine 5TGM1 and human JJN3 MM cells, **APX3330 (5-50μM) reduced Notch R3 mRNA expression by 50%, without affecting Notch R1, R2 or R4 levels, and decreased the expression of the Notch target gene Hes1 by 40%. Further, APX3330 decreased the number of viable MM cells by 80%, measured by MTT, and increased the number of dead MM cells in a dose-dependent manner, measured by trypan blue exclusion. These results suggest that Notch R3 is required to maintain Notch signaling and MM cell survival. In MLO-A5 osteocytic cells, APX3330 also downregulated the expression of Notch R3 by 45%, but not Notch R1, R2 or R4, and reduced the mRNA levels of the Notch target gene Hey1 by 60%. However, Ot viability and the number of dead Ot were not affected by APX3330, even when high concentrations were used. These results demonstrate that APX3330 effectively inhibits Notch signaling in both MM cells and Ot, and that induces MM cell death without altering Ot viability. Thus, APX3330 represents a novel pharmacologic approach to disrupt the bidirectional Notch signaling between Ot and MM and to inhibit Notch signaling in the MM microenvironment. These findings provide a basis for selective inhibition of Notch R3 signaling to treat MM patients and circumvent the deleterious side effects of systemic suppression of Notch using g-secretase inhibitors.**

Basic Science ***Graduate Student***

POSTER #12

DETERMINING THE ROLE OF ELF1 IN PROSTATE CANCER

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Various members of the ETS transcription factor family have been shown to undergo chromosomal rearrangements in prostate tissue and are involved in tumor formation and progression. The characterization of the roles for these oncogenes in prostate tissue is well studied; however, the functions of the normally expressed ETS proteins in prostate tissue are not well understood. Genomic and phenotypic assays, along with bioinformatic analyses, will be combined to gain insight into the role of ELF1 in the prostate. Preliminary results indicate that ELF1 is able to regulate key oncogenic phenotypes such as proliferation, survival, and migration in the prostate. Comparisons between the oncogenic ETS and ELF1 can be used to better understand the mechanisms of how the ETS family regulates oncogenic phenotypes and how more aggressive prostate tumors are formed.

Basic Science ***Graduate Student***

POSTER #13

ENZYME-IMMOBILIZED HYDROGELS FOR HYPOXIC IN VITRO CANCER CELL CULTURE

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Introduction: Hypoxia is an important physiological condition implicated in many healthy and diseased tissues in the body. For example, oxygen (O_2) concentration is around 20% in the lungs; ~13% in the alveoli; ~5% in the circulation system and the bone marrow; and below 5% in multicellular tissues including solid tumor. Given its role in regulating normal and pathological physiology, O_2concentration should be considered as a critical experimental condition when performing *in vitro* cell studies. This project aims at creating a hypoxic environment through enzymatic reactions. Here, we describe the development of glucose oxidase (GOX)-immobilized poly(ethylene glycol)-diacrylate (PEGDA) hydrogels to create solution hypoxia for up to 24hr under ambient conditions. The effects of enzyme-induced hypoxia were studied using Molm14 (acute myeloid lymphoma) as well as Huh7 (hepatocellular carcinoma) cancer cell lines. **Methods:** Macromer PEGDA (2kDa) and photoinitiator lithium arylphosphinate (LAP) were synthesized according to established protocols. Primary amine groups on GOX were modified with acrylate groups using acrylate-PEG-succinimidyl valerate. PEG-acrylate modified GOX (GOX_{PEGA}) was co-polymerized with PEGDA through UV-initiated photopolymerization to yield GOX-immobilized hydrogels. Both cell lines were seeded in 24-well plates. To allow time for attachment, Huh7 cells were cultured for 48hr prior to placement of GOX_{PEGA} gels. Molm14 cells were seeded and GOX_{PEGA} gels were placed immediately thereafter. Cobalt chloride ($CoCl_2$, a chemical stabilizer of hypoxia-inducible factor 1a (HIF1a) which simulates cellular hypoxic response) or catalase (CAT) was added as control groups. Percent O_2 was measured with an optical probe positioned 1-2mm above the gel (4-5mm from liquid-air interface). At select experimental times, cells were collected for RNA isolation, reverse transcription, and real-time PCR. **Results:** Efficacy of the enzyme system to induce hypoxia in the presence of cells was evaluated by monitoring O_2tension in the presence of cells. For GOX_{PEGA} gel groups, O_2 content dropped to ~8% within 1hr and was maintained below 5% for up to 24hr. In the presence of GOX-immobilized gel, expression of hypoxia associated gene, carbonic anhydrase 9 (CA9), was significantly upregulated for both Molm14 and Huh7 cells. For Huh7 cells alone, $CoCl_2$ failed to upregulate CA9 expression in the first 24hr compared to ~20-fold upregulation with GOX_{PEGA} gels. After 48hr of culture with Huh7 cells, $CoCl_2$ caused ~15-fold upregulation in CA9 expression, which was much lower than that induced by the GOX_{PEGA} gels (~80-fold higher). **Conclusions:** We have developed enzyme-immobilized hydrogels for inducing hypoxia for *in vitro* cell culture. The system was able to cause hypoxic cellular response for 24-48hr. Enzyme-immobilized hydrogels provide a simple and more efficient option to induce realistic hypoxia compared with chemical compounds. Future work will focus on developing O_2-gradients using hypoxia inducing enzyme-immobilized hydrogels.

Basic Science ***Graduate Student***

POSTER #14

JAK2 INDUCES LOCALIZATION OF MISMATCH REPAIR PROTEINS AND EPIGENETIC PROTEINS TO SITES OF OXIDATIVE DAMAGE

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Elevated levels of reactive oxygen species at sites of chronic inflammation cause oxidative DNA damage and contribute to tumorigenesis. Tumors have many aberrant epigenetic alterations, many of which affect the expression of tumor suppressor genes. Epigenetic changes occur at sites of chronic inflammation, however, it is not known how these epigenetic alterations are initiated. We hypothesize that the recruitment of epigenetic proteins to sites of oxidative DNA damage is important for initiating epigenetic alterations. Here we demonstrate that after hydrogen peroxide (H_2O_2) treatment, mismatch repair proteins MSH2 and MSH6 become localized to sites of oxidative DNA damage, resulting in the recruitment of epigenetic proteins such as DNA methyltransferase 1 (DNMT1) to chromatin and a concomitant reduction in expression of tumor suppressor genes. We sought to further understand the mechanism that drives H_2O_2-induced MSH2 and MSH6 chromatin binding. We demonstrate that (1) in response to H_2O_2 treatment, Jak2 interacts with MSH2/MSH6 in the nucleus, (2) Jak2 inhibition or knockdown reduces the H_2O_2-induced chromatin binding of MSH2, MSH6, and DNMT1, (3) Jak2 inhibition does not affect the H_2O_2-induced protein-protein interaction of MSH2 with MSH6 or DNMT1. These findings suggest phosphorylation of MSH2 and/or MSH6 by nuclear Jak2 may be important for their interaction with oxidative DNA damage. Understanding the mechanism by which MSH2, MSH6, and DNMT1 become localized to chromatin after oxidative damage will potentially allow us to develop treatment strategies to reduce the initiation of aberrant epigenetic alterations during chronic inflammation and therefore reduce tumorigenesis in people with chronic inflammatory diseases.

Basic Science ***Graduate Student***

POSTER #15

UNDERSTANDING EPIGENETIC DETERMINANTS OF RESPONSE TO PI3K TARGETED THERAPY IN PIK3CA DRIVEN BLADDER CANCER

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Background: Two bladder patient derived xenograft (PDX) models developed in our lab carry PIK3CA activating helical domain mutations. One model is responsive to treatment with dual PI3K/mTOR inhibitorLY3023414 (RP-B-02; PIK3CA E545K mutant) while the other model (RP-B-01; PIK3CA E542K mutant) is resistant. Interestingly, these two models are representative of distinct bladder cancer subtypes, where RP-B-01 is basal-like compared to RP-B-02 which is luminal. We hypothesize that distinct PIK3CA helical domain mutations (E545K and E542K) could play a potential role in driving resistance to therapy, driven by two mutually exclusive epigenetic loss of function mutations; KMT2D and KDM6A, which were detected in RP-B-01 and RP-B-02 respectively. Therefore we set out to compare the differential DNA methylation profiles between two models and investigate how it influences gene expression through protein-protein interaction (PPI) network associated with mutated genes detected in each model. The purpose is to identify potential players that drive resistance to treatment. **Methods**: WES coupled with RNA-seq was carried out to understand the molecular make up of our PDX models (RP-B-01 and RP-B-02). Phenotypic distinction of both models was confirmed using IHC, qPCR and WB for markers specific for luminal and basal subtypes (i.e. cytokeratins, p63, PPARG). A dual PI3K/mTOR inhibitor LY3023414 was used to assess drug response *in vivo* as well is *in vitro* using a novel 3D culture model. DNA methylation profile in both models was analyzed to determine its effect on gene expression. Analysis of PPI specific to mutated genes was performed to understand the role of mutation-leading epigenetic loss of function in driving phenotypic distinction in bladder cancer as well as differential response to treatment. **Results:** WES data proved both RP-B-01 and RP-B-02 models to be PIK3CA mutant, yet they carry distinct epigenetic loss of function mutations, KMT2D and KDM6A respectively. RNA-seq data coupled with confirmatory experiments showed that RP-B-01 is basal-like while RP-B-02 is luminal like. Our DNA methylation analysis recognized 14,944 differentially methylated regions (DMRs) ($q < 0.05$) locating within 4 kb upstream to 500 bp downstream of gene transcription start sites (TSSs). About one third (35.3%) DMRs were hypermethylated in B01 compared to B02. Interestingly; most differentially expressed genes (DEGs) (64.5%) associated with DMRs exhibited positive correlation between gene expression change and corresponding DNA methylation variation. However, DMRs negatively correlated with expression of associated genes were overrepresented in proximity to TSS. Importantly, PPI network of genes with none sense mutation specific to RP-B-01 and RP-B-02 (i.e. KMT2D and KDM6A respectively) were distinct with little overlap observed. They showed significantly different ratios of DEGs and DMRs, suggesting a potential role in driving phenotypic distinction and differential therapeutic response that is yet to be further investigated. **Conclusion:** Basal and luminal like bladder cancer carry distinct gene expression and DNA methylation profile. Although response to PI3K targeted therapy cannot be predicted by PIK3CA mutation, it could potentially be predicted by a distinct gene expression profile that is epigenetically driven in luminal and basal like bladder cancer.

Basic Science ***Graduate Student***

POSTER #16

INDUCTION OF OVARIAN CANCER STEM CELLS BY CANCER ASSOCIATED FIBROBLASTS LEADS TO CHEMORESISTANCE.

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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. Unlike other cancers, there has been little improvement in the 5-year survival rate of ovarian cancer patients over the past 3 decades. The standard of care involves cytoreductive surgery combined with adjuvant/neo-adjuvant carbo-taxol based chemotherapy. Most patients respond well initially but eventually experience relapse with platinum resistance, which is mainly lethal. Cancer stem cell theory can explain this phenomenon through the evidence of a persistent stem cell enriched population, which survives chemotherapy and causes relapse. Cancer stem cells have unique characteristics, some of which are shared with normal stem cells like regenerating themselves, differentiating into other cells, anchorage independent growth, enhanced DNA repair capacity, higher rate of membrane efflux, elevated expression of stem cell genes and markers and increased tumorigenicity. Various signaling pathways like TGF- ß, WNT, Hedgehog, JAK-STAT, PGDF and Notch are relevant to cancer stem cell thus a suitable microenvironment with relevant factors is essential for cancer stem cell maintenance. Cancer associated fibroblasts (CAFs) are one of the main constituents of the tumor microenvironment in ovarian tumors and can form 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.

Coculture of ovarian cancer cells with CAFs resulted in increased resistance to cisplatin, 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 cell population, which is a reliable marker for ovarian cancer stem cells. To identify if this increase of ovarian cancer stem cells was due to increased replication of existing cancer stem cells or though the induction of dedifferentiation of non-cancer stem cells into stem cells, we co-cultured FACS sorted ALDH negative cells with CAFs. The CAFs had the ability to turn non-cancer stem cells into cancer stem cells. This induction of cancer stem cells was even more enhanced when the cells were co-cultured in the presence of carboplatin. Treatment with conditioned medium from CAFs was sufficient to increase ALDH positive population and proliferation of ovarian cancer cells. Ovarian cancer cells treated with CAFs condition medium also demonstrated increased spheroid formation and chemoresistence, which are functional characteristics of cancer stem cells. Our results indicate that secreted factors from CAFs can induce ovarian cancer stem cells. Identification and targeting of these factors could be a novel therapeutic approach to treat chemoresistant ovarian cancer.

Basic Science ***Graduate Student***

POSTER #17

A NOVEL ROLE OF NF-KB INDUCING KINASE IN NEUROFIBROMATOSIS TYPE II

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Neurofibromatosis Type II (NF2) is an autosomal dominant cancer predisposition syndrome triggered by loss of heterozygosity at the *Nf2* locus. Characteristically, patients with NF2 suffer from tumors of neural crest derived origin, most commonly bilateral vestibular schwannomas as well as meningiomas, ependymomas, and astrocytomas. Because of their close proximity to the brain and brainstem, these tumors are often associated with significant morbidity and mortality. *Nf2* encodes the protein Merlin (Moesin-Ezrin-Radixin-Like Protein). Very little is known about the endogenous function of Merlin or how it functions as a tumor suppressor. In order to learn more about Merlin and how its loss leads to oncogenic transformation in select neural crest tissues, the lab generated a genetically engineered mouse (GEM) model of NF2. By adding Lox-P sites flanking exon two of the *Nf2* gene and expressing Cre recombinase driven by a 3.9 kb fragment of the *Periostin* promoter, we selectively ablated Merlin expression in neural crest progenitor tissues ($Nf2^{flox/flox}$;*Postn*-Cre). The resulting NF2 GEMs develop Schwann cell tumors and sensorineural hearing loss, accurately recapitulating the most common pathologies seen in NF2 patients.

In order to develop a deeper understanding for the global alterations in transcript levels and protein activity triggered by the loss of Merlin, we conducted a gene microarray on tumors from our NF2 GEM and as well as a whole kinome analysis on Merlin deficient cell lines and tumors from our NF2 GEM. We found both in cells and in tumor bearing tissues, NF-kB signaling was highly dysregulated in Schwann cells lacking Merlin. In particular NF-kB-inducing-kinase (NIK) appears highly overexpressed in human vestibular schwannomas as well as central and peripheral Schwann cell tumors in our mice. Using an adoptive cell transfer model we demonstrated that overexpression of a constitutively catalytically active fragment of NIK is sufficient to drive oncogenic transformation and Schwannoma formation in WT Schwann cells. Based upon this data I crossed $NIK^{flox/flox}$ mice onto our NF2 GEM background to test the hypothesis that loss of NIK will be sufficient to block oncogenic transformation and tumor formation in Merlin deficient Schwann cells. Historically, therapeutically targeting the NF-kB pathway has resulted in very poor outcomes in preclinical models due to significant on target toxicity. However, I have NIK germline deficient mice and they are viable, suggesting that at least in mice, NIK is dispensable for normal development and that the on target toxicity of inhibiting NIK pharmacologically should be manageable clinically. I am in the process of acquiring a NIK specific small molecule inhibitor which has been synthesized and is being developed as a cancer therapeutic. I hypothesize that this NIK inhibitor could be the first approved drug for the medical management of NF2.

Basic Science ***Graduate Student***

POSTER #18

RESTORATION OF MYC-REPRESSED MIR-29 IN PANCREATIC CANCER CELLS LEADS TO INCREASED REACTIVE OXYGEN SPECIES AND GEMCITABINE SENSITIZATION

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Pancreatic ductal adenocarcinoma (PDAC) still remains to be one of the most highly malignant human diseases and is resistant to the majority of current therapeutic modalities. Oncogene MYC has been known to be upregulated in PDAC and plays a key role in carcinogenesis and tumor progression. In our previous work, we found that downregulation of miR-29 is a common phenomenon of PDAC, and its restored expression reduced cancer cell migration/invasive potential, anchorage independent growth, and sensitized them to gemcitabine. Furthermore, we showed that miR-29 blocks autophagy in cancer cells by targeting key autophagy related proteins. However, the mechanism associated with the loss of miR-29 in PDAC has yet to be elucidated. Here we demonstrate that MYC inhibits miR-29 expression in PDAC. MYC nuclear localization negatively correlates with miR-29 expression in various pancreatic cancer cell lines, and knockdown of MYC led to an increased expression of both primary miR-29a/b1 transcript and mature miR-29a. Furthermore, miR-29 overexpression in combination with gemcitabine led to decreased cancer cell viability and increased intracellular reactive oxygen species (ROS) and cell death. Our findings show for the first time that MYC represses miR-29 in PDAC, and miR-29 sensitizes pancreatic cancer cells to gemcitabine through increased intracellular ROS. Taken together our results indicate the potential use of miR-29a as a novel therapeutic agent for PDAC.

Basic Science ***Graduate Student***

POSTER #19

ENZYME-MEDIATED STIFFENING HYDROGELS FOR STUDYING PANCREATIC CELL MALIGNANCY

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Introduction: Pancreatic cancer has extremely poor prognosis and current therapeutic strategies have not yielded significant improvement in patient survival rate. The intense stromal reactions in pancreatic cancer have been suggested to play a key role in tumor progression. It is also believed that the behaviors of the cancer cells are partially affected by matrix stiffness, potentially through an epithelial-mesenchymal transition (EMT) process. To mimic a stiffening tumor as *in vivo*, we present a biomimetic dynamic hydrogel by using norbornene (NB), hydroxyphenylacetic acid (HPA) dually functionalized gelatin, which was crosslinked by thiol-modified hyaluronic acid (THA) through orthogonal thiol-norbornene photopolymerization. The additional HPA moieties permit tyrosinase-mediated dopachrome formation, which led to *in situ* gel stiffening. The stiffening HA/Gelatin hybrid gels were used to study pancreatic cancer malignancy in three-dimension (3D). **Methods:** Gelatin-norbornene (GtNB) was synthesized as described previously and it was further conjugated with HPA through carbodiimide chemistry to yield dually functionalized GtNB(HPA). Desired amounts of GtNB or GtNB(HPA) (7 wt%), as well as thiolated hyaluronic acid (THA) (2 wt%) and photoinitiator LAP were mixed and irradiated under 365nm light (5 mW/cm^2) for 2 minutes. Hydrogels were stored in PBS (37°C)for two hours, followed by incubating in tyrosinase solution for 6 hours to achieve in situ stiffening. Gel elastic moduli (G' & G") were measured by oscillatory rheometry in strain-sweep mode. COLO-357 cells were used as a model to evaluate the influence of matrix stiffening on cancer cell fate. Cells (2 million cells/mL) were encapsulated in gels composed of GtNB or GtNB(HPA) with THA. In situ stiffening of cell-laden hydrogels was performed one day post-encapsulation. Live/dead staining and confocal imaging were performed to evaluate cell viability and morphology. **Results:** ^1H NMR analyses results demonstrated successful modification of gelatin into GtNB and GtNB(HPA). The crosslinking of GtNB(HPA) and THA yielded biomimetic hydrogels susceptible to tyrosinase-mediated *in situ* stiffening. To demonstrate this, we prepared GtNB-THA and GtNB(HPA)-THA hydrogels with initial moduli of around 1,000 Pa. After tyrosinase treatment, gel moduli increased 3-fold for GtNB(HPA)-THA hydrogels and the high stiffness maintained for several days. These gels were highly cytocompatible and the encapsulated COLO-357 cells were able to proliferate over a two-week culture period. More importantly, we observed significant cell spreading in stiffened gels, but not in soft gels. Specifically, cells formed condensed clusters when encapsulated in hydrogels that were not susceptible to stiffening (i.e., GtNB-THA). On the other hand, cells were spread out significantly in the stiffened GtNB(HPA)-THA hydrogels, potentially due to a stiffness-induced EMT process.

Basic Science ***Graduate Student***

POSTER #20

NOVEL SERINE 176 PHOSPHORYLATION OF YBX1 ACTIVATES NF-¿B IN COLON CANCER

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The Y box protein 1 (YBX1) is a well-known oncoprotein which has tumor promoting functions. YBX1 is widely considered to be an attractive therapeutic target in cancer. In order to develop novel therapeutics to target YBX1, it is of great importance to understand how YBX1 is finely regulated in cancer. Previously, YBX1 has been shown to function as a tumor promoter through phosphorylation of its serine 165 (S165) residue, leading to the activation of the NF-[kappa]B signaling pathway. Here we show our discovery of a quite distinct phosphorylation site, S176, on YBX1. Overexpression of the YBX1-S176A (serine to alanine) mutant in either HEK293 cells or colon cancer HT29 cells shows a dramatically reduced NF-[kappa]B activating ability as compared with that of WT-YBX1, confirming that S176 phosphorylation is critical for the activation of NF-[kappa]B by YBX1. Importantly, the mutant of S176 and the previously reported S165 sites regulate distinct groups of NF-[kappa]B target genes, suggesting the unique and irreplaceable function of each of these two phosphorylated serine residues. Our important findings could provide a novel cancer therapeutic strategy by blocking either S176 or S165 phosphorylation or both of YBX1 in colon cancer.

Basic Science ***Graduate Student***

POSTER #21

THE ROLE OF THE CHROMATIN MODIFIER, LSD1, IN THE PRO-TUMORIGENIC TRANSCRIPTIONAL RESPONSE TO INFLAMMATION.

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Inflammatory bowel disease (IBD) represents a critical risk factor for the development of colorectal cancer (CRC) and on-going inflammation also drives the progression of CRC. Inflammation and the associated oxidative damage can alter the activity of histone modifiers, and we have begun to connect inflammation to changes in the activity of the histone lysine-demethylating enzyme LSD1. LSD1 is overexpressed in approximately 80% of CRC and in early and late stage cancer tissues, suggesting it may be involved in cancer initiation and progression. Overexpression of LSD1 promotes proliferation and metastasis in part by aberrant regulation of critical epithelial-mesenchymal transition (EMT) genes in colon cancer cells. Our preliminary data indicate that LSD1 plays a role in the transcriptional response to treatment of cells with the reactive oxygen species hydrogen peroxide. We determined that LSD1 transcriptionally represses expression of extracellular matrix factors, cell-adhesion molecules, and immune response genes both basally and in response H_2O_2. The current focus of our work is to determine direct transcriptional targets of LSD1 basally and after oxidative damage with a goal of understanding the mechanism by which LSD1 mediated repression is altered at sites of inflammation. This study aims to advance our understanding of LSD1 as a therapeutic target in colon cancer and provide a framework for new treatment modalities against this disease.

Basic Science ***Graduate Student***

POSTER #22

STROMAL MMP3 INHIBITS ONCOGENIC POTENTIAL DURING BREAST CANCER PROGRESSION

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The tumor microenvironment is composed of multiple cell types and extracellular proteins that interact with breast cancer epithelium. Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that regulate the microenvironment around epithelial cells. MMPs degrade extracellular matrix proteins to promote tumor invasion and to regulate signaling pathways. MMP3 functions during both breast development and cancer progression. We assessed MMP3 protein localization in both normal and tumor mammary tissue by immunohistochemistry. During puberty, MMP3 protein localized to epithelial ductal cells, adipocytes, and other stromal cells. During development, MMP3 expression decreased and was largely absent during pregnancy and lactation. In contrast, MMP3 protein became expressed again during involution and localized to the mammary epithelial cells and to some stromal cells. Over the course of development, MMP3 cellular localization and intensity changed according to the mouse age and developmental process.

While auto-activation and overexpression of MMP3 in mammary epithelium has been shown to induce hyperplasias and tumor formation, the differential roles in the breast of stromal and epithelial MMP3 have not been distinguished. To determine the requirement for MMP3 in breast cancer, we transplanted cancer cells (PyMT) into the mammary glands of MMP3 knockout (KO) and heterozygous (Het) syngeneic mice. Mammary tumor burden increased in MMP3 KO mice compared to Het, suggesting an inhibitory role for stromal MMP3. H&Es of primary tumors show similar pathologies between the MMP3 Het and KO tumors. However, immunohistochemistry analysis showed increased cell proliferation and increased immune cell infiltration but no difference in apoptosis between the Het and KO tumors. We also discovered that stromal MMP3 was required for visible lung metastases. To determine the step of metastasis that requires stromal MMP3, we injected cancer cells into tail veins of MMP3 Het and KO mice. *In vivo* imaging and quantification of a luciferase reporter gene, counting of visible metastasis, and pathological analysis determined that MMP3 KO mice developed increased lung metastasis compared to Het mice. This suggests that stromal MMP3 in the lungs inhibits metastatic tumors. Together, our data suggest that stromal MMP3 has a protective role during breast cancer development and metastasis.

Basic Science ***Graduate Student***

POSTER #23

UNDERSTANDING THE FUNCTION OF THE ONCOGENE ERG FOR TARGETED SMALL MOLECULE INHIBITION

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For men in the United States, prostate cancer is the second most common cancer and the second leading cause of cancer-related death. 50% of prostate cancers are caused by a chromosomal rearrangement that results in expression of the oncogenic transcription factor ERG. To specifically target ERG with therapeutics, we needed a better understanding of the mechanisms of ERG function in prostate cells. We recently identified a co-activator, EWS, that specifically interacts with ERG and is required for oncogenic ERG functions. We are currently optimizing an AlphaScreen to identify small molecule inhibitors of the ERG-EWS interaction as a way to target ERG function in prostate cancer. Additionally, we are investigating the functional mechanisms of the ERG/EWS complex. Preliminary data suggest that in addition to activating transcription, ERG and EWS can also regulate the length of target mRNA. This is important because transcripts with varied sequence at the 3' end can have differential responses to miRNA regulation, altered transcript stability, and altered translation efficiency. ERG targets that promote cell migration were found to undergo alternative polyadenylation, producing shortened transcripts. The length of these targets were restored upon EWS knockdown. Serine 2 phosphorylation (Ser2P) of the C-terminal domain (CTD) of RNA Polymerase II peaks at the 3' end of genes to allow for the proper assembly of polyadenylation factors. ERG expression in normal prostate cells results in the aberrant enrichment of Ser2P of the CTD at 5' ends of genes that undergo proximal polyadenlyation usage. This effect was lost at genes that undergo distal polyadenylation usage. This suggests that ERG and EWS stimulate Ser2P at targets important for cancer progression, to allow for early recruitment of 3' processing factors, resulting in shortened isoforms. These data are the first to show ERG functioning in a co-transcriptional process.

Basic Science ***Graduate Student***

POSTER #24

ABERRANTLY EXPRESSED MICRORNAS DRIVE THE DEVELOPMENT OF ACQUIRED ERLOTINIB-RESISTANCE IN NON-SMALL CELL LUNG CANCER

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Lung cancer is the third most prevalent cancer in the world, however it is the leading cause of cancer related deaths worldwide. Non-small cell lung cancer (NSCLC) accounts for ~85% of the lung cancer cases, with aberrations in some common signaling pathways. The current strategies to treat NSCLC patients with causal genetic mutations is through targeted therapeutics. Approximately 10-35% of NSCLC tumors confer activated mutations in Epidermal Growth Factor Receptor (EGFR) resulting in uncontrolled cellular proliferation. The standard-of care for such patients is Erlotinib, a therapeutic that inhibits EGFR activity. However, most Erlotinib treated NSCLC patients acquire Erlotinib Resistance (ER) within a year post treatment. Acquired ER in patients can be attributed to either a secondary mutation in EGFR or activation of alternative pathways, while in 15-20% of cases, the molecular mechanisms remain unidentified. As multiple mechanisms are involved in ER, here we speculate that microRNAs(miRNAs), which are largely unexplored, may function as drivers of acquired ER in NSCLC.

To this end, we propose an unbiased study either (i) overexpressing, or (ii) knocking-out miRNAs in Erlotinib sensitive cells with the hypothesis that perturbations in miRNA levels will drive Erlotinib resistance. Therefore, the Erlotinib dose response curves of two Erlotinib sensitive NSCLC cell lines, EKVX and H322M were established. For the miRNA overexpression screen, EKVX cells stably expressing Renilla and Firefly luciferase genes were generated (EXVX-RFGLO) to monitor cell number and transfection efficiency, respectively. Further, EKVX-RFGLO cell response to 75% of growth inhibitory concentrations of Erlotinib (GI-75) was determined, and the time point to observe resistance evaluated using two positive controls – miR-21 and antisense-17 (AS17). Next, 2,019 individually arrayed human miRNAs will be transfected in EKVX-RFGLO cells, and cell growth in the presence of Erlotinib will be monitored. In the knock-out screen, we aim to identify miRNAs, that when lost, will drive Erlotinib resistance. For this, EKVX and H322M cells stably expressing Cas9 protein were generated and characterized. To obtain full coverage of the lentiviral expressed small guide RNA (sgRNA) library, targeting 20,914 genes (including 1,864 miRNA genes), EKVX-Cas9 cells were transduced at 400-fold coverage. Cells were passaged in both GI-75 and GI-90 concentrations of Erlotinib for 10 passages, DNA isolated, sgRNAs PCR amplified, libraries generated and deep sequenced to identify the target genes critical for Erlotinib response. For sgRNAs that were significantly more abundant in Erlotinib treated samples, relative to untreated samples, bioinformatic analysis was performed. Specifically, sgRNAs targeting protein coding genes such as receptors including G-proteins, and sgRNAs targeting miRNA genes that regulate important cellular processes like cell cycle were identified. Genes of interest will be verified via knock-down and overexpression strategies in EKVX, H322M-Cas9 cells, and Erlotinib resistance will be confirmed.

Basic Science ***Graduate Student***

POSTER #25

ELONGATION CONTROL OF RNA POLYMERASE II TRANSCRIPTION BY TFIIS

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Transcription by RNA Polymerase II (RNAPII) requires strict regulation in order to maintain proper function. Inability to properly regulate gene expression or deal with issues that occur during transcription can be catastrophic for a cell and can lead to genome instability and disease. A key event that occurs during transcription elongation is RNAPII pausing. Pausing has been implicated in the control of diverse processes including: transcriptional fidelity, elongation, RNA splicing, and termination. There are many causes of RNAPII pausing such as DNA damage, well-positioned nucleosomes, DNA binding proteins, and so on that can cause RNAPII to stop RNA synthesis. Resolution of pausing by either resumption of elongation or termination that removes RNAPII from the DNA is necessary for proper gene expression control. We propose that there are multiple mechanisms used to deal with paused RNAPII. These mechanisms include the action of elongation factors that can keep RNAPII associated with the DNA during pausing and provide other activities that allow transcription to resume. The focus of this work is to investigate the role of the transcription elongation factor Dst1 (SII, TFIIS) in the rescue of RNAPII pausing following backtracking. Backtracking occurs when RNAPII moves backwards on the DNA template resulting in a lack of a 3' hydroxyl in the active site, which is required for nucleotide addition and extension of the nascent RNA. Dst1 is known to stimulate the intrinsic nuclease activity of RNAPII to facilitate backtracked RNA cleavage and the resumption of RNAPII elongation. In humans, TFIIS has been shown to play a role in definitive hematopoiesisas well as cancer cell proliferation. Our central hypothesis is that perturbation of *DST1* will lead to the accumulation of paused RNAPII throughout the genome. In this study, we perturbed *DST1* in the model organism *Saccharomyces cerevisiae* and measured changes in RNAPII DNA occupancy, the composition of the RNAPII interactome (+/- TFIIS), and RNAPII post translational modifications (+/- TFIIS). Our results show global changes in RNAPII occupancy at protein coding genes but not at sn/snoRNAs. These data suggest that Dst1 is required to maintain RNAPII elongation of protein coding genes. We are testing the effects of Dst1 deletion on the RNAPII interactome by LC-MS/MS. Subsequently, SAINT analysis will be performed to determine the statistical significance of the changes as a result of *DST1* perturbation. The findings of these experiments will help provide a better understanding of the resolution of RNAPII stalling during transcription elongation. Further investigations of the mechanisms that resolve paused RNAPII will not only broaden our understanding of transcription elongation and termination, but may also give us insight into degradation of RNAPII, RNAPII proofreading, and DNA damage repair.

Basic Science ***Graduate Student***

POSTER #26

INVESTIGATING THE ROLE OF EPIGENETIC ENZYME PROTEIN ARGININE METHYLTRANSFERASE 5 AS AN ONCOGENE AS WELL AS A THERAPEUTIC TARGET IN GASTROINTESTINAL CANCERS

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The class of gastrointestinal cancers including pancreatic ductal adenocarcinoma (PDAC) and colorectal cancer (CRC) are notoriously challenging for treatment. Hyperactive nuclear factor kB (NF-kB), an important eukaryotic transcription factor, is a common culprit in both cancers. Previously, we discovered that the epigenetic enzyme protein arginine methyltransferase 5 (PRMT5) dimethylated and activated NF-kB. This was a significant finding since increased activation of NF-kB and its downstream target genes is a common feature of both PDAC and CRC, and is not only associated with poor disease prognosis but also linked to developing resistance against chemotherapy. Here, we show that PRMT5, the epigenetic activator of NF-kBis highly expressed in PDAC and CRC. Overexpression of PRMT5 promoted cancer progression, while shRNA knockdown showed an opposite effect, at least partly via regulating NF-kB activity. Using an innovative AlphaLISA high throughput screen, we discovered a lead compound that inhibited PRMT5 activity, namely PR5-LL-CM01, which exhibited robust tumor inhibition effects in both cancers. An *in silico* structure prediction suggested that PR5-LL-CM01 inhibits PRMT5 by binding with its active pocket. Importantly, PR5-LL-CM01 showed anti-tumor efficacy in both PDAC and CRC mouse models. We also demonstrated that PR5-LL-CM01 treatment led to a decrease in NF-kB activation in PDAC and CRC cell lines. Significantly, PR5-LL-CM01 showed higher efficacy than the commercial PRMT5 inhibitor, EPZ015666 in both PDAC and CRC. Overall, this study highlights the critical role of the epigenetic enzyme PRMT5 to drive NF-kB activation in the context of PDAC and CRC. Furthermore, our work clearly highlights the significant potential of PRMT5 as a therapeutic target in PDAC and CRC and establishes PR5-LL-CM01 as a promising basis for new drug development in the future.

Basic Science ***Graduate Student***

POSTER #27

ROLE OF CXCL5 CHEMOKINE IN BREAST CANCER DORMANCY IN AN EX VIVO BONE CULTURE MODEL

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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 also detrimental to the patient's quality of life by causing hypercalcemia, acid/base imbalance, aberrant hematopoiesis, immune response, and osteolysis (bone loss). These symptoms increase fracture risk, resulting in severe pain and immobility. Current treatment options for patients with metastatic bone cancer include combinations of treatments such as chemotherapy, palliative radiation, and surgical resection. However, bone metastasis is not considered curable with current therapies. Circulating tumor cells sometimes become arrested in blood vessels or within tissue, remaining in a quiescent state ('dormancy') until the right conditions induce the cancer cells to grow and thrive in the metastatic site ('colonization'). Switching cancer cells from dormancy to colonization is rate-limiting for bone metastasis, which colonization sometimes taking decades to induce metastatic tumor growth. With few experimental models available to study this last step of metastasis, the switch from cancer dormancy to colonization has become one of the greatest challenges in cancer treatment and cancer research. To model dormancy, we developed an ex vivo culture system of mouse bones and cancer cells. Using our culture system, we 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. Additional studies using CXCL5 recombinant protein and inhibitors of CXCL5 further suggest that CXCL5 contributes to breast cancer dormancy in metastatic bone disease.

Basic Science ***Graduate Student***

POSTER #28

BMI1 LOCALIZATION TO 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; high density of which is found in 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 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 hypothesize that BMI1, a member of Polycomb repressive complex 1 could potentially be involved in transcriptional repression of key genes by H2A ubiquitination, contributing to cisplatin resistance. BMI1 mediated ubiquitination of H2A has been shown to occur at sites of double strand breaks. During development and cell differentiation, ubiquitination of H2A by BMI1 is known to be involved in gene silencing. Our preliminary data demonstrates H2A ubiquitination and BMI1 co-localization with ¿H2AX after cisplatin treatment. Furthermore candidate genes that are known to be silenced by DNA methylation in cisplatin resistant cells, are transcriptionally repressed following acute cisplatin treatment. The central hypothesis for my project is that when cells are treated with cisplatin, damage occurs in the promoter CpG islands of genes causing recruitment of BMI1 and other repair proteins to sites of damage. This recruitment occasionally causes stable gene silencing ultimately contributing to the development of cisplatin resistance.

Basic Science ***Graduate Student***

POSTER #29

ERK PHOSPHORYLATION OF THE ONCOGENIC TRANSCRIPTION FACTOR ERG IN PROSTATE CELLS DISSOCIATES EZH2 ALLOWING TRANSCRIPTION ACTIVATION

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A chromosomal rearrangement that results in fusion an androgen driven promoter to the open reading frame of the transcription factor ERG is observed in ~50% of prostate cancers. ERG, which is not normally expressed in prostate epithelium, promotes cell migration, epithelial-to-mesenchymal transition, and angiogenesis. ERG's transcriptional activity is known to be modified by numerous post-translation modifications as well as interactions with various co-activators and co-repressors. However, the exact mechanism of ERG's transcriptional activation is not known. Our lab has previously demonstrated that activated Ras/MAPK signaling is required for ERG's function in prostate cells, however, the molecular mechanisms of this requirement are not known.

Here we demonstrate that sequential phosphorylation of ERG by ERK1/2 results in a conformational change in ERG and subsequent dissociation of the repressor EZH2, resulting in transcriptional activation. Utilizing *in vitro* and *in vivo* biochemistry, ChIP-Seq, and RNA-Seq we show that failure to phosphorylate ERG resulted in recruitment of EZH2 across the ERG-cistrome and loss of transcriptional activation. Further, we demonstrate that phosphorylation of ERG by ERK1/2 is necessary for tumor growth in a mouse xenograft model. Next, we sought to explore the mechanisms by which EZH2 represses ERG dependent transcription as well as the epigenetic changes associated with ERG expression. Preliminary data demonstrates that ERG expression results in differential recruitment of EZH2 to target genes and alters H3K27me3 distribution across the cistrome.

Overall these data demonstrate a molecular mechanism ERG's activation and oncogenic activity. Therefore, these findings could guide therapeutic intervention in ERG-positive prostate cancer.

Basic Science ***Graduate Student***

POSTER #30

EPIGENETIC TARGETING OF DNMT1 IN ADIPOCYTES INHIBITS HIGH-GRADE SEROUS OVARIAN CANCER CELL MIGRATION AND INVASION THROUGH SUSD2 UPREGULATION

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Approximately 22,440 women were diagnosed with ovarian cancer in 2016 and the five-year survival rate is only 28.3%, making ovarian cancer the deadliest gynecologic disease. Ovarian cancer frequently metastasizes to the omentum and adipocytes play a significant role in tumor progression. A recent meta-analysis that included 25,157 women indicated that increased risk of ovarian cancer is accompanied by increased weight.As methylation levels in obese adipose tissue are increased due to increased DNMT1 levels and activity, it was of interest to test the hypothesis that inhibiting DNMT1 would reverse adipocyte methylation, alter adipokine secretion, and decrease migration and invasion of ovarian cancer cells towards adipocytes.

The presence of adipocytes increases migration and invasion of ovarian cancer cells in Boyden chamber assay, verifying previously published reports. Proximal co-culture of adipocytes increases proliferation of Kuramochi and OVCAR4 cells. Direct coculture of adipocytes and OVCAR8-RFP cells increases carboplatin resistance of OVCAR8-RFP cells. Treatment of adipocytes with DNMT inhibitor (100nM guadecitabine) significantly decreased migration and invasion of SKOV3, Kuramochi, OVCAR4, OVCAR8, and OVCAR5 cells towards adipocytes Also, guadecitabine-treated adipocyte conditioned media decreased EMT markers in OVCAR4 and OVCAR8 cells.

The known mechanism of guadecitabine is cleaved decitabine incorporates into DNA in a replicating cell and will covalently entrap DNMT1, leading to DNMT degradation. However, mature adipocytes do not divide. The possibility of preadipocytes in the cell culture dish was deemed null due to the absence of preadipocyte marker, *DLK1*, mRNA expression and presence of adipocyte marker PPAR¿. Also, BrdU incorporation was undetectable in mature adipocytes as assessed by immunocytochemistry. In order to determine the mechanism of action of guadecitabine in adipocytes, subcellular protein fractionation of adipocytes treated with guadecitabine revealed DNMT1 degradation even in the presence of DNA synthesis inhibitor, aphidicolin.

Methyl-capture sequencing and RNA-sequencing of guadecitabine-treated adipocytes reveals significant demethylation and reexpression of genes involved in cytokine signaling and inhibition of matrix metalloproteases. Integrated analysis of significantly demethylated genes and significantly reexpressed genes from the sequencing data sets produced a gene of interest, SUSD2. SUSD2 is a secreted protein that is known mainly as a tumor suppressor in colon, hepatocellular, non-small cell lung, renal cell, and high-grade serous ovarian cancer. The effects of DNMT1 inhibitors on mature adipocytes are vastly unknown but this is the first study to show that guadecitabine treatment of adipocytes decreases migration and invasion of ovarian cancer cells possibly though reexpression of SUSD2.

Basic Science ***Graduate Student***

POSTER #31

NON-CELL AUTONOMOUS EFFECTS OF PTPN11E76K CONTRIBUTE TO MPN IN A MURINE MODEL OF JMML

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Juvenile Myelomonocytic Leukemia is a pediatric myeloproliferative disorder (MPN). JMML is caused by clonal somatic mutations in RAS-ERK signaling genes, including PTPN11, which evoke growth hypersensitivity to GM-CSF in hematopoietic progenitors due to hyperactive *RAS* signaling. JMML patients present with monocytosis, anaemia, hepatosplenomegaly, and have a disheartening 52% survival following allogenic stem cell transplantation—the only curative treatment.

JMML has been studied in mice expressing PTPN11^{E76K} using the Cre-loxP system. Existing models, however, have crucial shortcomings. They drive oncogene expression in non-hematopoietic cells, which inappropriately exacerbates disease features. Additionally, they simultaneously induce expression in >90% of blood cells and thus do not recapitulate the clonal origins of JMML. In this study, we set out to create an improved model of this disease. We hypothesized that CSF1R-MCM+;ROSA26$^{YFP/+}$;PTPN11$^{E76K/+}$ (henceforth CSF1R-Cre;PTPN11^{E76K}) would demonstrate defining features of JMML due to non-cell autonomous effects of low frequency hematopoietic-restricted PTPN11^{E76K} expression.

We assessed the activity of the CSF1R-Cre by measuring YFP expression one week after tamoxifen injection. 2.2% of BM HSCs and 1.7% of splenic HSCs were YFP+. Importantly, YFP expression was not observed in endothelial cells, mesenchymal cells, and osteoblasts, indicating the hematopoietic specificity of CSF1R-Cre. We generated CSF1R-Cre;PTPN11^{E76K} mutant animals and confirmed that only YFP+ cells efficiency recombined the PTPN11^{E76K} locus. We proceeded to monitor the peripheral blood of mutant and CSF1R-Cre;PTPN11$^{+/+}$ littermates for 52 weeks. Compared to controls, mutant animals had an 8-fold increase in %YFP+ cells. These YFP+ mutant cells were preferentially CD11b+, indicating the myeloproliferative effects of PTPN11^{E76K} expression. Upon histological examination, mutants had marked hepatosplenomegaly and their frequency of YFP+ BM and splenic progenitors was up to 10-fold higher than in controls. Amazingly, YFP- cells were also affected. They showed biased differentiation to CD11b+ myeloid cells in the blood, BM, and spleen. Additionally, we observed a relative decrease in BM HSCs and an increase in splenic HSCs, a pathognomonic feature of extramedullary hematopoiesis and MPN.

In functional tests, mutant progenitors produced greater number of colonies at low doses of GM-CSF compared to controls. Additionally, both YFP+ and YFP- cells from mutants had increased frequencies of pERK+ cells at 5, 10, and 30min after GM-CSF stimulation compared to controls. To assess the cause, we repeated these assays with sorted YFP+ and YFP- progenitors from mutant animals. Amazingly, isolated YFP- cells demonstrated the same GM-CSF hypersensitivity and RAS hyperactivation as YFP+ cells. Finally, in long-term follow-up CSF1R-Cre;PTPN11^{E76K} animals succumbed to MPD with a median survival of 70 weeks.

In summary, our findings highlight that low frequency hematopoietic-restricted PTPN11^{E76K} expression is sufficient for MPN development because it comprises the normal hematopoiesis of WT progenitors that co-

habit the mutant niche. Our results have broad implications in the pursuit of treatment strategies for hematopoietic malignancies.

Basic Science ***Graduate Student***

POSTER #32

THE ROLE OF DAB2IP IN OVARIAN CANCER STEM CELLS

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Ovarian cancer is a leading cause of death from gynecologic malignancies. The majority of women diagnosed with advanced-stage epithelial ovarian cancer experience lethal tumor relapse after initial therapy of carboplatin and paclitaxel. Failure to eliminate ovarian cancer stem cells (OCSCs) by the end of conventional chemotherapy is considered a main reason for tumor relapse and metastasis.

DAB2IP, a novel member of RasGTPase-activating (GAP) protein family, was reported to inhibit many aspects of cancer progression and was downregulated in many human cancers. In addition, loss of DAB2IP in prostate and colon cancer was shown to promote CSCs like features, suggesting its critical role in modulating CSCs survival.

In this study, we aim to elucidate the tumor suppressor role of DAB2IP in regulating OCSCs and explore the epigenetic approaches to upregulate its expression. We observe higher DAB2IP expression in non-CSCs compared to OCSCs. DAB2IP overexpression reverses stemness-related phenotypes, including reduced ALDH activity, decreased spheroid and colony formation ability, and decreased expression of genes correlated with stemness, such as OCT4, NANOG, TWIST and etc. Furthermore, elevated DAB2IP expression in OCSCs decreases cisplatin IC50 and fewer cells migration in transwell assay. As aberrant DNA promoter methylation and histone modification are major regulators of DAB2IP expression in cancer, DAB2IP expression is increased after DNMT (guadecitabine) and EZH2(GSK-126) inhibition, accompanied by reduced OCSCs. Our data suggests a possible role of DAB2IP in modulating CSC-driven ovarian cancer progression in vitro, potentially upregulated by epigenetic approaches.

Basic Science ***Graduate Student***

POSTER #33

A NON-CYTOTOXIC COMPOUND BLOCKS ANGIOGENESIS AND DECREASES TUMOR BURDEN IN THE TAG-RB RETINOBLASTOMA MOUSE

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Purpose: Cytotoxic systemic or local chemotherapy is a mainstay of retinoblastoma therapy but is associated with significant side effects. Antiangiogenic therapy could avoid some of this toxicity. We developed the novel antiangiogenic compound SH-11037, which likely acts as a soluble epoxide hydrolase inhibitor. SH-11037 blocks angiogenesis in vitro and in murine ocular neovascularization models, making it a candidate for antiangiogenic retinoblastoma therapy. Here, we explored the effects of SH-11037 on retinoblastoma cells in culture and as a treatment for retinoblastoma in the TAg-RB transgenic mouse model.

Methods: We assessed the effects of SH-11037 on proliferation of Y79 retinoblastoma cells and ARPE-19 "normal" retinal pigment epithelial cells by alamarBlue proliferation assays. For therapeutic evaluation, TAg-RB mice were intravitreally injected with SH-11037 or vehicle to a final vitreous concentration of 10 μM weekly starting at 7 or 8 weeks of age with sacrifice and enucleation at 10 or 11 weeks. We followed tumor progression by optical coherence tomography in vivo and histopathological analysis post-mortem. Percent tumor burden in each eye was compared between cohorts and treatments by two-way ANOVA.

Results: SH-11037 did not block the growth of retinoblastoma or retinal pigment epithelial cells in culture, suggestive of a lack of toxicity. However, in transgenic mice with retinoblastoma, SH-11037 significantly reduced tumor burden over vehicle (up to 26% reduction, $p=0.022$). No adverse effects were observed with weekly compound injections.

Conclusions: SH-11037 is not toxic to tumor or healthy cells of the eye, but when administered by intravitreal injection may be a candidate for retinoblastoma treatment by blocking angiogenesis. Further validation of the possibility of intravitreal, antiangiogenic retinoblastoma therapy using other drugs and other preclinical models is indicated.

Basic Science ***Medical Student***

POSTER #34

THE UTILIZATION OF MOLECULAR THERAPY TO TARGET THE C-KIT/KIT LIGAND PATHWAY IN NEUROFIBROMATOSIS TYPE 1

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Neurofibromatosis Type 1 (NF1) is a common genetic tumor predisposition syndrome that results in malignant and non-malignant neoplasms throughout the neuroaxis. NF1 is caused by mutations in the *Nf1* tumor suppressor gene, which encodes the protein neurofibromin. Neurofibromin functions as a GTPase activating protein (GAP) for p21 Ras. Loss of both *Nf1* alleles in the tumor-initiating cells results in constitutive activation of Ras and its downstream effectors, driving tumor formation. One of the most common tumors affecting people with NF1 is the plexiform neurofibroma (pNF). These are complex, slow-growing tumors that cause major morbidity and mortality through impingement on vital organs and propensity for malignant transformation. Genetic data in preclinical murine models of NF1 implicate a key role of the c-Kit/Kit ligand pathway in tumor initiation and early progression. We hypothesized that preventing mast cell recruitment or degranulation at the tumor site by targeting the c-Kit/Kit ligand pathway could impact *Nf1*-mediated tumorigenesis.

Herein, utilizing genetically engineered mouse models (GEMMs) of robust NF1 pNFs, we have tested ketotifen and imatinib mesylate - two pharmacological agents that target c-Kit/Kit ligand-mediated mast cell functions - and evaluated their impact on pNF formation and growth.

Ketotifen is an FDA-approved mast cell stabilizer. A preliminary, non-randomized clinical trial dating back to the 1980s tested the potential of ketotifen to impact tumor burden and a small subset of patients reported decreased growth rate of their cutaneous and plexiform tumors. In our preclincal mouse model, we tested ketotifen's hypothesized impact on Kit-mediated mast cell functions, along with its impact on reducing established pNF tumor burden and preventing tumor formation. Our results demonstrate that when used with and without continuous, provocative Kit ligand infusion, ketotifen reduced the number of degranulating mast cells but not the mast cell infiltrate measured in skin. Importantly, ketotifen did not influence pNF formation or size.

Imatinib mesylate, an inhibitor of various tyrosine kinases including c-Kit, demonstrated the first objective evidence of tumor shrinkage in a subset of pre-existing pNFs in a phase 2 clinical trial. However, the opportunity to prevent pNF formation with imatinib mesylate had not yet been formally tested. Therefore, in our pNF GEMM, we evaluated imatinib mesylate's impact on preventing tumor initiation. Our results indicate that early, low-dose imatinib mesylate is sufficient to prevent pNF genesis. Further, even after the cessation of preventative imatinib mesylate treatment and prolonged follow up, the size of residual tumors was significantly reduced as compared to age-matched vehicle controls.

Collectively, these data demonstrate that ketotifen is not sufficient to correct all c-Kit/Kit ligand-mediated functions nor is it sufficient to prevent tumor initiation or reduce established tumor burden. These data do support the use of imatinib mesylate as a preventative therapeutic for plexiform neurofibromas.

Basic Science ***Medical Student***

POSTER #35

PROMOTION OF OVARIAN CANCER METASTASIS BY MICROENVIRONMENT-INDUCED ETS1 UPREGULATION.

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A key step in the process of abdominal dissemination in ovarian cancer (OC) is the attachment and subsequent establishment of colonies at the site of metastasis. Paracrine/juxtacrine interactions with the microenvironment of the metastatic site are critical for metastasis and lead to altered gene expression essential for colonization. Transcription factors (TFs) play a major role in regulation of gene expression and several important TF families have been implicated in OC. In particular, the ETS family of TFs has been shown to promote cellular migration and invasion through the activation of genes such as matrix metalloproteases. However, the identity of the ETS factors that mediate OC metastatic colonization, and the role of the tumor microenvironment in this function are unclear. Using an organotypic 3D culture model recapitulating the early events of metastasis, we identified ETS1 as the most upregulated member of the ETS family of TFs in the metastasizing OC cells. We also found an increased ETS1 expression in human ovarian cancer samples as compared to normal fallopian tubes using a tissue microarray. Moreover, higher expression of ETS1 was a predictor of poor prognosis in OC patients. Knocking down ETS1 decreased migration, proliferation and colony formation as well as invasion through and colonization of the organotypic 3D culture. Overexpression of ETS1 had the opposite effect. CRISPR/Cas9-mediated knockout of ETS1 resulted in decreased tumor burden in mouse xenografts. A combination of ChIP-seq and RNA-seq analysis revealed that ETS1 promoted an EMT phenotype and FAK was one of the major transcriptional targets. Inhibition of FAK functionally mimicked the effects of ETS1 inhibition in the OC cells. Moreover, rescue experiments established FAK as a downstream effector of ETS1 during OC metastasis. ETS1 itself was found to be upregulated by secreted factors from mesothelial cells covering the omentum. The interaction with the mesothelial cells activated MAP kinase signaling, which activated ETS1 and increased its transcription. Taken together, our results indicate that ETS1 is a key transcription factor induced by the microenvironment in the metastasizing OC cells. The increased expression of ETS1 is a result of secreted factors from mesothelial cells covering the omentum, which activate the MAP kinase pathway in the OC cells. ETS1 promotes OC metastatic colonization though the transcriptional upregulation of its target FAK.

Basic Science ***Medical Student***

POSTER #36

USE OF APERIO WHOLE SLIDE DIGITAL IMAGING AND IMAGE ANALYSIS FOR PRE-CLINICAL TRIAL STUDIES

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Aperio Imaging provides customizable imaging techniques that are becoming an industry standard today. Image analysis with Aperio has the ability to quantify area and cells by identifying colors according to stain and intensity in order to quantify. Aperio can perform whole slide digital scanning that takes approximately 2.5 minutes per slide and can capture data for imaging in the 2-40x range. Aperio Imaging can be used with H&E, Trichrome, and PAS stains. Data is generated using the Aperio positive pixel software using algorithms to assign percentages to levels of positive staining in comparison to the entire area. Analysis can be completed using the entire image or by choosing hotspot areas (typically three to five) that accurately represent the tissue as a whole. In this study, aging mouse tissue samples were evaluated using Aperio positive pixel software to analyze percent tumor (lymphoma) in kidney and lung H&E slides. In conclusion, the positive pixel algorithm was successful in identifying tumor cells using H&E slides.

Aperio Imaging provides customizable imaging techniques that are becoming an industry standard today. Image analysis with Aperio has the ability to quantify area and cells by identifying colors according to stain and intensity in order to quantify. Aperio can perform whole slide digital scanning that takes approximately 2.5 minutes per slide and can capture data for imaging in the 2-40x range. Aperio Imaging can be used with H&E, Trichrome, and PAS stains. Data is generated using the Aperio positive pixel software using algorithms to assign percentages to levels of positive staining in comparison to the entire area. Analysis can be completed using the entire image or by choosing hotspot areas (typically three to five) that accurately represent the tissue as a whole. In this study, aging mouse tissue samples were evaluated using Aperio positive pixel software to analyze percent tumor (lymphoma) in kidney and lung H&E slides. In conclusion, the positive pixel algorithm was successful in identifying tumor cells using H&E slides.

Basic Science ***Pathology Intern***

POSTER #37

CHARACTERIZATION OF A DEWAR-OXETANE SPECIES FORMED DURING A PYRIMIDINE PHOTOREACTION.

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Pyrimidine (6-4) pyrimidone photoproduct (6-4 PP) is one of the major DNA photolesions generated under UV-irradiation and is widely believed to be a major contributor to DNA mutations in the skin and subsequent occurrence of skin cancer. 6-4 PP is believed to be formed via a short-lived oxetane intermediate by Paterno-Buchi reaction between the C5=C6 of the 5'-thymine and the C4=O of the 3' thymine. Characterization of the putative oxetane intermediate in pyrimidine photochemistry has not been achieved due to its extremely high instability. We replaced the 5'-thymine residue with a xylene moiety(X) to generate a dinucleotide analog XpT. UV irradiation of this XpT produces a 6-4PP analog. Interestingly, upon further irradiation, this 6-4PP analog is converted to a relatively stable Dewar-oxetane species that is stable enough to be isolated by reverse-phase HPLC and characterize by NMR spectroscopy at -25 °C. Additionally, the Dewar-oxetane formation reaction is reversible: it can be readily converted back to the 6-4 PP under 254 nm UVC light.

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

POSTER #38

INFLAMMATION INDUCED FUNCTIONAL CHANGES IN PRE-LEUKEMIC STEM AND PROGENITOR CELLS LACKING TET2 CAN BE MODULATED BY TARGETING THE NFKB PATHWAY

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ABSTRACT

While recent studies have begun to shed light on the impact of inflammation on the function of normal hematopoietic stem and progenitor cells (HSPCs)[1,2], little is known about how these processes are regulated in pre-leukemic HSPCs. Here, we have investigated the response of normal and Tet2-deficient pre-leukemic HSPCs and mature myeloid cells to acute inflammation. We show that, although the hematological counts in the peripheral blood of both wildtype and *Tet2*-knockout (*Tet2*-KO) mice return to normal levels at later stages of inflammation, granulopoiesis and activation of HSPCs is significantly extended during early stages of inflammation in these mice relative to controls. This difference is associated with a significant increase in the production of pro-inflammatory cytokine IL-6, enhanced genomic instability and progenitor cell survival in *Tet2*-KO mice compared to controls. Functionally, Tet2-deficient stem cells demonstrate resistance to inflammatory challenge as revealed by a higher repopulating and engraftment potential in both primary and secondary recipients compared to wildtype controls, which, when stressed, show a remarkable decline in overall engraftment. Mechanistically, Tet2-deficient HSPCs demonstrate elevated expression of TLR4/NFkB pathway components under both naïve and inflammatory conditions. E3330, an Ape1 inhibitor that blocks redox signaling activation of NFkB, and recently approved for phase I clinical trial for malignancies in solid tumors, partially reversed the extended inflammatory phenotype seen in Tet2-deficient mice. Collectively, this study provides insight into the selection advantage that might render Tet2-deficient HSPCs susceptible to transformation upon inflammation and suggest that anti-inflammation therapy could be of clinical benefit for normal individuals carrying *TET2* mutations that show signs of clonal hematopoiesis as well as those patients with *TET2* mutations in blood cells such as patients with acute myeloid leukemia, myeloproliferative disease and myelodysplastic syndrome.

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

POSTER #39

INVESTIGATING AGING AND OVARIAN CANCER METASTASIS SITES WITH QUANTITATIVE MASS SPECTROMETRY-BASED PROTEOMICS OF MURINE ADIPOSE TISSUE

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The risk of developing ovarian cancer increases with age. Poor prognosis is associated with age, and younger patients tend to have lower tumor burden. A murine model of aging and ovarian cancer metastasis demonstrated that aged mice (20-23 months) are more susceptible to intraperitoneal metastasis than young mice (3-6 months). Gonadal adipose tissue from aged mice was found to have greater tumor burden than young mice. To investigate possible protein factors involved, we analyzed adipose tissue from the periovarian fat depots from healthy non-tumor bearing young and aged mice with high-resolution quantitative mass spectrometry-based proteomics. Adipose tissue is a poorly understood and underutilized tissue group for proteomics and requires specialized sample preparation. The presence of large amounts of lipid complicates trypsin digestion and chromatography for adipose tissue. We utilized a simple sample clean-up procedure adapted for whole tissue lysis, with centrifugation and precipitation to delipidate the samples. A gel-based proteomic procedure provided pre-fractionation and contaminant removal prior to UPLC-nESI-MS/MS analysis. Label-free quantification was performed using MaxQuant's Label Free Quantification (LFQ) algorithms. We anticipate the analysis will provide insights into preferential colonization of aged periovarian tissue in a relevant murine model of ovarian cancer metastasis. Elucidation of protein drivers in the metastatic niche may provide targets for therapy and advances in differential care for elderly women with metastatic disease.

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

POSTER #40

HUMAN HEMATOPOIETIC STEM CELL HOMING AND ENGRAFTMENT PROMOTED BY GLUCOCORTICOID INDUCED CHROMATIN REMODELING

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Efficiency of hematopoietic stem cell (HSC) homing is important for enhanced hematopoietic cell transplantation (HCT), especially when numbers of HSCs are limited as with cord blood (CB). The SDF-1/CXCR4 chemotactic axis is a major pathway directing migration and homing of HSC from peripheral blood to bone marrow niches. To get enhanced insight into achieving better homing efficiency of human CB HSC, we performed a compound screen to search for small molecules which could enhance surface expression of CXCR4 and SDF-1/CXCR4 axis mediated chemotaxis. From a nuclear hormone ligand library including 74 chemical compounds, we found that treatment of CB $CD34^+$ cells with Dexamethasone, a synthetic glucocorticoid (GC), greatly promoted surface expression of CXCR4. Expression of CXCR4 on CB $CD34^+$ cells was also increased after treating cells with other glucocorticoids (not existing in the library) including Flonase, Cortisol, and Medrol. We focused on use of Flonase, the compound which forms the most stable activated complex with glucocorticoid receptor (GR) (Högger, P. & Rohdewald, P. Steriods, 1994), and which enhanced CXCR4 expression at concentrations as low as 10 nM. Pretreament of CB $CD34^+$ cells with Flonase significantly increased surface CXCR4 expression and promoted SDF-1/CXCR4 axis mediated chemotaxis of human CB HSC. Further more, Flonase pretreatment rigorously enhanced homing and long-term engraftment of phenotypically-defined human CB HSC and progenitor cells (HPC).

Mechanistically, GC pretreatment significantly increased mRNA and protein levels of CXCR4 in human CB $CD34^+$ cells. A glucocorticoid response element (GRE), AGAACATTCTGTGCA was found in the CXCR4 promoter region (from -1662 bp to -1648 bp upstream of the transcription start site). Chromatin immunoprecipitation analysis suggested that GR was remarkably enriched in this region of the CXCR4 promoter upon administration of GC. CXCR4 promoter activity was enhanced by Flonase stimulation, and this was abrogated by deletion of the GRE. Co-immunoprecipitation analysis demonstrated that the SRC1/p300 complex which has histone acetyltransferase activity was recruited to the CXCR4 promoter by GR upon Flonase treatment. Acetylation levels of CXCR4 associated Histone 4 on Lysine 5 and Lysine 16 (H4K5 and H4K16) were dramatically enhanced by Flonase treatment, while acetylation levels of Histone 3 (H3K9 and H3K14) remained unchanged. Enhanced chemotaxis and homing of Flonase treated CB HSC and HPC was totally blocked by administration of p300 inhibitor. Our findings indicate that short-term pretreatment with glucocorticoid hormone dramatically improves CB HSC homing and engraftment by selective chromatin remodeling. This suggests a simple, new, and easily adaptable means to enhance clinical efficacy of CB HCT.

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

POSTER #41

ROLE OF RAB11B-MEDIATED ENDOSOMAL RECYCLING DURING BRAIN METASTATIC OUTGROWTH

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Breast cancer brain metastases are an urgent clinical problem, accounting for 30% of breast cancer mortality. Tragically, as targeted therapies provide better control of localized and systemic disease, women who respond to initial treatment ultimately develop brain metastases, and currently, no clinically approved drug shows promising efficacy for brain metastases. It is well established that communication with the metastatic microenvironment is critical for metastatic success, but the exact nature of this communication is poorly understood. We hypothesize that genes up-regulated during the progression from single cells to actively proliferating brain metastases are functionally required for metastatic progression. To dissect the dynamic nature of brain metastatic progression, we performed transcriptome analysis in small tumors 7 days post injection (dpi) and overtly metastatic tumors 40 dpi. We identified 108 of the most highly up-regulated genes, and utilized a *Drosophila* model to screen 448 RNAi constructs for a functional role in brain metastasis. We identified Rab11 as an important mediator of brain metastatic outgrowth. Rab11 regulates the specificity of vesicular transport in the endosomal and exosomal recycling pathways, and is responsible for recycling of surface proteins back to the surface. Examination of Rab11b mRNA levels in human breast cancer cells reveals that Rab11b is moderately up-regulated in primary tumors, but very strongly up-regulated in brain metastases. Indeed, Rab11b is necessary for the formation of breast cancer brain metastasis. Interestingly, Rab11b is most highly expressed at 6 dpi, when cells have successfully adapted to the brain microenvironment and initiated metastatic outgrowth. Further, the brain microenvironment is dramatically different from the breast, and cells are forced to adapt to this new metabolic environment to survive and form successful metastases. We find that glia afford protection to breast cancer cells, allowing them to survive the metabolic stress of low glucose/low glutamine culture conditions. Rab11b is required for this protection, suggesting a role for recycling in metabolic adaptation. Together, our findings indicate that breast cancer cells up-regulate Rab11b as they adapt to the brain microenvironment, and this up-regulation is required for successful progression from single cells to overt metastases.

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

POSTER #42

NEUTRALIZING NEGATIVE EPIGENETIC REGULATION BY HDAC5 ENHANCES HUMAN HAEMATOPOIETIC STEM CELL HOMING AND ENGRAFTMENT

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Allogeneic haematopoietic cell transplantation (HCT) is a lifesaving therapy to treat patients with haematologic disorders and cancer. Human cord blood (CB) contains a lifesaving source of haematopoietic stem cells (HSCs) and progenitor cells (HPCs) for transplantation. CB transplantation has many advantages including reduced need for HLA matching, ready availability, lower incidence of GvHD and lower risk of transmissible virus infection. However, limited numbers of HSC/HPC or poor homing are problematic for efficient CB HCT. Here we report that specific HDAC5 inhibition highly upregulates CXCR4 surface expression in human CB HSCs and HPCs. LMK235, a selective inhibitor of HDAC5, increased membrane CXCR4 expression in CB $CD34^+$ cells. In contrast, inhibition of other HDACs did not show any effect on membrane CXCR4 expression. LMK235 treatment resulted in significantly higher CXCR4 mRNA, membrane CXCR4 expression in CB HSCs, enhanced migration to SDF-1a in chemotaxis assay, and higher number of cells homed to BM environment in NSG mice. We next performed chromatin immunoprecipitation (ChIP) assays to examine the chromatin status at the CXCR4 promoter region. H3K9 and H4K16 acetylation levels were significant higher in LMK235-treated CB $CD34^+$ cells compared with vehicle control, suggesting increased H3K9 levels at the CXCR4 promoter region contributes to increased CXCR4 transcription. To further explore the mechanisms underlying HDAC5 regulation of HSC homing, we tested a couple of signaling pathways and found that inhibition of the NF-¿B signaling pathway by Andrographolide and BMS345541, suppressed LMK235-mediated CXCR4 upregulation on CB $CD34^+$ cells. It has been reported that the NF-¿B signaling pathway regulates CXCR4 expression in breast cancer cells and acetylation of NF-¿B p65 subunit enhances its transcriptional activity. We examined the acetylation levels of p65 and found that LMK235 treatment resulted in increased levels of p65 acetylation in CB $CD34^+$ cells, indicating p65 could be a downstream target of HDAC5. Consistently, using ChIP assay we detected increased levels of acetylated p65 binding to the CXCR4 promoter region in the LMK235-treated group compared to vehicle control. Furthermore, activation of the NF-¿B signaling pathway via TNFa also resulted in significantly increased CXCR4 surface expression and enhanced HSC homing. These results demonstrate that neutralizing a previously unknown negative epigenetic regulation of HSC homing and engraftment by HDAC5 and NF-¿B signaling allows for a new and simple translational strategy to enhance HSC transplantation. Since higher expression of CXCR4 by cancer cells is associated with metastatic migration, our study also suggests that it might be clinically applicable to inhibit tumor cell migration by targeting HDAC5.

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

POSTER #43

GENERATION OF IMMORTALIZED BREAST EPITHELIAL CELL LINES FROM NORMAL BREAST REPRESENTING INTRINSIC SUBTYPES OF BREAST CANCER

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ABSTRACT:

Breast cancers are classified into five intrinsic subtypes based on comparative gene expression analyses between tumors. These include luminal subtype A, luminal subtype B, HER2-positive, basal, and normal-like. There is ethnicity-dependent variation in incidence of and/or outcome from these intrinsic subtypes. For example, basal breast cancers with higher degree of intratumor heterogeneity and worst outcome are more prevalent in African American women compared to Caucasian women. It is unknown whether differences in normal breast biology contribute to ethnicity-dependent differences in outcome. In addition, it is also unknown whether normal breast contain cells representing intrinsic subtypes. Using normal breast tissue from the Komen Tissue Bank, we have previously demonstrated enrichment of unique PROCR(CD201)+/EpCAM- multi-potent progenitor cells in the normal breast of African American women compared to Caucasian or Hispanic women. The goal of this study was to create resources to study ethnicity-dependent differences in normal breast biology and to test whether non-transformed cell lines representing different intrinsic subtypes can be created from the normal breast. Using a primary cell culturing system that provides a replenishable source of normal epithelial cells of different lineages including estrogen receptor-positive luminal, luminal progenitors, and stem cells from different ethnic groups, we created 10 telomerase-immortalized breast epithelial cell lines from normal breast of ancestry-mapped Caucasian, African American and Hispanic women. RNA sequencing and PAM50 intrinsic subtype clustering algorithms were used to identify the intrinsic subtypes of the immortalized cell lines. Our cell lines represented 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 double positive for KRT 14 and KRT 19, but in varying proportions. We further characterized these cell lines for distribution patterns of stem, luminal progenitor, mature/differentiated and multi-potent PROCR (CD201)-positive progenitor cells using CD44/CD24, CD49f/EPCAM and CD201/EPCAM cell surface markers and flow cytometry. Stem/progenitor/differentiation state varied from cell line to cell line. We also observed widespread variation in the mRNA expression levels of lineage-restricted hormonal signaling network genes including estrogen receptor alpha, GATA3 and FOXA1. NF-¿B, which is involved in cancer progression, particularly in hormone receptor negative breast cancers, showed variable DNA binding activity. Collectively, resources developed in this study can be used to dissect 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 #44

REGULATION OF THE FUNCTIONAL PROPERTIES OF XERODERMA PIGMENTOSUM COMPLEMENTATION GROUP A (XPA) PROTEIN THROUGH LYSINE ACETYLATION

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Nucleotide Excision Repair (NER) is the main repair pathway through which DNA damages occurring on account of ultraviolet (UV) or ionizing radiation (IR) are repaired. Xeroderma pigmentosum complementation group A (XPA) protein is a crucial part of the NER multi-protein complex that plays an indispensable role in recognizing and binding the damaged DNA, as well as positioning other NER proteins optimally for efficient repair of the injury. Studies have shown that XPA can be dynamically acetylated and deacetylated impacting its involvement in the NER repair pathway. Deacetylation of XPA by SIRT1 enhances its interaction with the single strand DNA (ssDNA) binding protein, replication protein A (RPA). Our current research is focused on evaluating the effects of lysine acetylation on the structural and functional properties of XPA.Our mass spectrometry analysis revealed that XPA is *in vitro* acetylated at multiple sites by p300 and CBP acetyltransferases. We evaluated the effects of acetylation on the DNA binding properties of XPA protein using electrophoretic-mobility shift assays (EMSA) and bio-layer interferometry (BLI) technology. Interestingly, our results show that acetylation of XPA significantly lowers its binding affinity for cisplatin damaged DNA. Similar to reports *in vivo*, analysis of protein-protein interactions between XPA and RPA, revealed that acetylation of XPA reduces its ability to interact with RPA. Our results suggest that acetylation of XPA negatively affects its role in recognizing, binding and consequently repairing damaged DNA, which in turn may impact subsequent recruitment of RPA. By application, our results present a mechanistic approach towards negatively modulating the effectiveness of the NER pathway, with the aim of improving the efficacy of current chemotherapeutics.

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

POSTER #45

DISRUPTION OF THE ESTRADIOL-REGULATED NTN1-UNC5A DEPENDENCE RECEPTOR SIGNALING AXIS CAUSES A HYBRID BASAL/LUMINAL MOLECULAR PHENOTYPE IN ESTROGEN RECEPTOR-POSITIVE BREAST CANCER CELLS

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Luminal subtype of breast cancers that express the estrogen receptor alpha (ERa) represents approximately two-thirds of all breast cancer cases. ER^+ tumors tend to have the most favorable prognoses when treated with endocrine therapy. However, a relapse or endocrine therapy resistance is often seen in ER^+ breast tumors. UNC5A belongs to the dependence receptor family which can mediate two different intracellular signals: cell survival, differentiation or migration when engaged with its ligand (such as Netrin-1; NTN1) or cell death/apoptosis in the absence of the ligand. Here we demonstrate that, depending upon the cell type, *UNC5A* and *NTN1* are estradiol (E2)-inducible genes. Using shRNA or CRISPR knockdown strategies, we show that the disruption of the NTN1-UNC5A signaling axis in ER^+ (MCF7 and T-47D) cells generates a mixed basal-like/luminal phenotype with stem cell-like characteristics. RNA-seq of *UNC5A* knockdown cells showed deregulated expression of several E2-target genes in both cell lines. Moreover, knockdown of *UNC5A* resulted in increased cell proliferation, and elevated expression of the E2-inducible anti-apoptotic, BCL2. Furthermore, the expression of *¿Np63* was enhanced in *UNC5A* knockdown cells. *¿Np63* isa TP53 family transcription factor that promotes breast epithelial stem cell maintenance and basal-like breast cancer. Accordingly, *UNC5A* knockdown cells displayed cancer stem cell phenotype as evident from ~3-fold increase in the number of $CD44^+/CD24^+$, $CD44^+/EPCAM^+$ and $ITGA6^+/EPCAM^+$ subpopulation compared with control cells. In addition, the expression of *NTN4* increased upon *UNC5A* knockdown. NTN4is a ligand that enhances permeabilization and remodeling of lymphatic vessels. In vivo, *UNC5A* knockdown cells implanted in nude mice were able to form tumors in the mammary fat pad independent of E2 supplementation and were able to colonize and develop into overt metastasis in multiple organs such as lungs, ovaries and adrenal glands. Consequently, analysis of mammary fat pad tumors from animals that received *UNC5A* knockdown cells revealed an increased expression of PECAM1 (CD31), a marker for endothelial cells used to evaluate tumor angiogenesis. In contrast to *UNC5A*, knocking down *NTN1*,decreased the expression of BCL2 and TP63 in both cell lines. Thus, knockdown of *UNC5A* resulted in deregulated expression of E2-regulated genes, E2-independent and anti-estrogen-resistant growth in vitro, and E2-independent tumor formation in xenograft models. Consistent with results of in vitro studies, analysis of tissue samples from breast cancer patients (n = 196) revealed that lower expression of UNC5A is associated with lower overall survival ($P < 0.05$). Thus, loss or mutational inactivation of *UNC5A* could lead to unrestricted E2:ERa signaling while simultaneously enabling ERa-positive luminal breast cancer cells to acquire basal-like and cancer stem cell-like features.

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

POSTER #46

ROLE OF PROTEIN TYROSINE PHOSPHATASE SHP2 IN A MOUSE MODEL OF AML DRIVEN BY LOSS OF DNA METHYLTRANSFERASE, TET2 IN COMBINATION WITH FLT3-ITD

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Background: SHP2 is an essential protein that acts as a node to integrate signals from several different tyrosine kinase receptors with the intracellular signaling pathways such as ERK, PI3K and STAT pathways to regulate cell survival, proliferation and differentiation. One such cytokine receptor kinase, FLT3 with internal tandem duplication (ITD) is constitutively active and has been reported to co-operate with loss of Tet2 to transform myeloproliferative neoplasm (MPN) to acute myeloid leukemia (AML) in mouse models. These mouse models are physiologically relevant as these two mutations are frequently found in combination in AML patients and similar to them show increase in leukemic stem cells and resistance to conventional chemotherapy as well as FLT3 targeted kinase inhibitor therapy.

Aims: Inhibition of SHP2 is crucial for relaying the signals from Flt3 and its inhibition has been reported to retard the growth of receptor tyrosine kinase driven malignancies; we wanted to see if loss of of SHP2 will inhibit AML development and progression in $Tet2^{-/-}Flt3^{ITD/+}$ mouse models of AML.

Methodology: We generated $Ptpn11^{F/F}Tet2^{-/-}Flt3^{ITD/+}Mx1Cre^{+}$ or $Ptpn11^{F/F}Tet2^{-/-}Flt3^{ITD/+}Mx1Cre^{-}$ mice through a series of crosses and Ptpn11 was deleted at 8-10 week of age using poly IC. Cells from mice with or without deletion of Ptpn11 were also used in transplantation model to assess leukemia initiation and cell autonomous effects upon Ptpn11 deletion. Changes in the hematopoietic compartment were analyzed by flow cytometry.

Results: The median survival of $Ptpn11^{F/F}Tet2^{+/-}Flt3^{ITD/+}Mx1Cre^{+}$ was significantly altered along with WBC counts post poly IC treatment as compared to $Ptpn11^{F/F}Tet2^{+/-}Flt3^{ITD/+}Mx1Cre^{-}$ mice (n=8). The Ptpn11 deleted leukemic mice also showed almost complete loss of long term HSC with increase in short term HSC in the bone marrow. However, they were still able to engraft and initiate leukemia in lethally irradiated recipient mice. Both primary and transplanted mice with deletion of Ptpn11 in the context of $Tet2^{-/-}Flt3^{ITD/+}$ had impaired ability to generate mature B cells but no such defects in myeloid compartment. The effects of Ptpn11 deletion were more severe in primary mice as compared to mice that received Ptpn11 deleted cells or when Ptpn11 was deleted after transplantation.

Conclusions: Our results demonstrate that the role of SHP2 is dependent upon the presence of other genetic mutations. SHP2 regulates AML in this model of loss of Tet2 with concomitant expression of Flt3-ITD through influence on both leukemic cells and the bone marrow microenvironment.

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

POSTER #47

UCHL1 IS A NOVEL MEDIATOR OF HIGH-GRADE SEROUS OVARIAN CANCER METASTATIC PROGRESSION AND PROGNOSIS

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Ovarian cancer is the most lethal of all gynecological malignancies accounting for 14,240 deaths annually in the United States. High-grade serous ovarian cancer (HGSOC) is the most prevalent and aggressive sub-type of ovarian cancer. About 70-80% of ovarian cancer patients with HGSOC tumors have low survival rates. Detection at advanced stages when patients present with metastatic tumors and subsequent development of chemo-resistant tumors greatly contributes to the high-mortality rate associated with HGSOC. The initiation and development of HGSOC is known to proceed through the acquisition of genetic alterations in the tumor suppressor gene TP53. However, the precise mechanisms that drive HGSOC progression remain unclear. Thus, it is critical to understand the pathobiology of HGSOC to identify therapeutic target or biomarker of HGSOC progression and prognosis.

We observed overexpression of deubiquitinase UCHL1 (ubiquitin carboxyl terminal hydrolase 1) in HGSOC cell lines and tissue microarray of ovarian cancer patients. High UCHL1 levels were associated with poor survival of ovarian cancer patients. We next studied UCHL1 expression in 9 pairs of HGSOC primary tumors and normal fallopian tube tissue (from the same patient). UCHL1 expression was significantly high in 8 out of 9 primary tumors compared to their fallopian tube controls. Interestingly, about 64% (9 out of 14) of metastatic tumors showed elevated UCHL1 levels compared to the primary tumors. Similarly, a HGSOC mouse model exhibited high UCHL1 levels in primary vs. metastatic murine tumors compared to normal fallopian tube controls. These results indicate that UCHL1 levels in HGSOC increase with cancer progression. To investigate the role of UCHL1 in HGSOC progression, we stably knocked down UCHL1 in HGSOC cell lines. Supporting our observation, silencing UCHL1 significantly reduced cancer cell proliferation and migration. Our mass spectrophotometry results identified FAK (focal adhesion kinase) as one of the target of UCHL1. Silencing UCHL1 significantly reduces FAK phosphorylation and FAK levels. Together, these results suggest that UCHL1 promotes HGSOC tumorigenesis possibly via focal adhesion kinase.

This study was supported by IUSM Biomedical Research Grant.

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

POSTER #48

CXCL-12 EXPRESSION BY TUMOR STROMA IS RESPONSIBLE FOR RECRUITMENT OF PLASMACYTOID DENDRITIC CELLS IN MALIGNANT GLIOMA

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Background: Glioblastoma (GBM) is highly proliferative, infiltrative and most lethal type of primary malignant brain tumor with poor prognosis and is the biggest therapeutic challenge of the modern era. GBM is characterized by dense immunosuppression induced by the tumor microenvironment and is the biggest hurdle to overcome in designing effective anti-glioma immunotherapy. Plasmacytoid dendritic cells (pDCs) are bone marrow derived antigen presenting cells (APCs) that are enriched in glioma microenvironment early during tumor formation. pDCs are known to express CXC receptor 4 (CXCR4), which binds to the chemokine CXCL-12. In various other cancers, CXCL-12 has been shown to be a potent regulator of pDC migration.

Hypothesis: Thus, we hypothesize that CXCL-12 expression by glioma is responsible for pDC recruitment in the glioma microenvironment.

Methods: In this study, using the orthotropic, syngeneic, and immunocompetent mouse model of glioma, we analyzed the role of CXCL-12 in pDC recruitment. C57BL/6 mice were intracranially injected with GL261/CXCL-12_{over}, GL261/CXCL-$12_{knockdown}$ and $GL261_{vc}$ cells. After one week post operation (wpo) and three wpo, tumor tissue was isolated and analyzed by flow cytometry for pDC populations. In addition, mice were also followed for a survival study.

Result: Our results show, that GL261 cells secrete CXCL-12 at baseline. CXCL-12 concentration significantly increased in GL261 over expression cell as compared to GL261 control cell. Flow cytometric analysis showed a higher percentage of pDCs in the tumor microenvironment at 1wpo in mice bearing CXCL-12 overexpressing glioma compared to the control group. In addition, the frequency of pDCs were lower in the CXCL-12 knockdown group compared to vector control. Mice bearing CXCL-12 overexpressing glioma had significantly shorter survival compared to the control group. Increased pDCs in the tumor microenvironment resulted in an increased frequency of CD8+CD28-T cells in the tumor bearing mice.

Conclusion: In conclusion, we show that CXCL-12 secreted by glioma cells recruit pDCs in the tumor microenvironment. Increased pDCs in the tumor results in shorter survival of tumor bearing animals. pDCs also expand the population of CD8+CD28-T cells.

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

POSTER #49

DEVELOPMENT OF A INFORMATICS PIPELINE FOR LABEL-FREE AND TANDEM MASS TAGGED-BASED QUANTITATIVE PROTEOMICS

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Mass spectrometry (MS) based proteomics is a highly quantitative approach that can be used to measure an array of biological changes including total protein and post-translational modification (PTM) levels. To incorporate new approaches in protein quantitation, we have developed an informatics pipeline using Proteome Discoverer 2.1 and Scaffold Q+. To illustrate the application of these software tools, we have performed both label-free and tandem mass tagged (TMT) quantitative experiments to explore options for analysis and visualization of highly complex datasets. Overall, we have found that both Proteome Discoverer and Scaffold Q+ have unique advantages for proteomics dataset analysis. Proteome Discoverer has an integrated quantitative method for MS/MS-based TMT quantitation for up to 10 different samples per MS run. In addition, the Research Institute of Molecular Pathology has produced a number of open-source nodes for Proteome Discoverer 2.1 including a label-free MS1 intensity quantitative tool known as Peakjuggler and a PTM localization tool known as ptmRS. After peptide-spectrum matching is performed in Proteome Discoverer 2.1 using SEQUEST-HT, the data can be opened in Scaffold Q+. Scaffold software includes stylized MS/MS spectrum views that facilitate manual investigation of fragment ions. TMT-based quantitation can also be interrogated within Scaffold Q+ to generate publication quality figures that illustrate the quantitative values measured for proteins and specific peptides. Overall, we have established a robust analysis pipeline for both label-free and isotope labeled-based quantitative proteomics data.

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

POSTER #50

IMMUNOLOGICAL AND GENE EXPRESSION PROFILE OF SPONTANEOUS CANINE GLIOMA

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Background: Glioblastoma (GBM) still remains one of the deadliest form of brain tumors in humans. Although, there's been much insight into the tumor microenvironment and possible treatments are in development, an effective therapy still hasn't been developed. Mouse models are critical in progressing research for glioma, but they fail to capture the complexity of GBM in human patients. Human gliomas are highly heterogeneous and complex tumors that arise spontaneously in immunocompetent hosts. Comparatively, companion dogs also spontaneously develop malignant gliomas with clinical and histopathological features similar to human glioma.

Hypothesis: Thus, we hypothesize that spontaneous canine glioma model is a superior translational model to demonstrate the molecular and immunological characteristics of human glioma.

Methods: In this study, we used flow cytometry, RNA *in situ* hybridization and genomic sequencing to characterize the immune landscape of canine glioma. We defined various T cell and dendritic cell populations in terms of their activation status and in addition, we analyzed oncogenes important to drive tumor progression in canine glioma. Finally, we compared our findings with the human glioma data to validate the canine glioma model.

Results: Compared to normal brain, canine glioma samples had higher CD8+, CD4+ and CD4+FoxP3+ regulatory T cell infiltration. These findings parallel literature describing T cell infiltration in human glioma. Similar to human patients, peripheral blood of canine glioma patients showed higher expression of the inhibitory surface receptor, PD-1, on CD4+ and CD8+ T cells. Canine gliomas also show similar gene expression profiles as in human gliomas, e.g. PDGFRA, Olig2 and CDK4. The pathways most altered in canine gliomas, e.g. the cell cycle, TGF-ß signaling and angiogenesis, are also similar to human gliomas.

Conclusion: In conclusion, our results demonstrate that spontaneous canine glioma resembles human gliomas very closely in terms of immune infiltrate and gene expression profiling. This model provides a great translational model to test therapies proven successful in mouse model prior to transitioning to human clinical trials.

Basic Science ***Research Technician***

POSTER #51

CDKN2A/B PLAYS AN IMPORTANT ROLE IN TRANSITION FROM PERIPHERAL NEUROFIBROMA TO MPNST.

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Ras activation induces broad physiological processes by transmitting pro-growth signals to multiple downstream proteins. In pathological conditions chronic RAS activation also induces activation of compensatory proteins that inhibit cell cycle progression and induce apoptosis or senescence. .. Specifically, in some established cancers, such as colon cancer and malignant melanoma, oncogenic Ras induced INK activation serves as a protective mechanism of pathological cell growth by causing cellular senescence. INK4 is a tumor suppressor-signaling network that is associated with CDKN2A/B, and is responsible for the regulation of the cell cycle and apoptosis through Rb, and P53. However, the role that it plays in decreasing the tumor size or slowing growth in other malignancies, including peripheral nerve sheath tumors is incompletely understood. Our hypothesis is RAS mediated oncogenic tumor growth is leading activation of INK4A/B tumor suppressor, and that loss of the tumor suppressor is sufficient to allow progressive tumor transformation. CDKN2A/B may play an important role in transition from plexiform neurofibroma to atypical neurofibroma and eventual progression to MPNST; by targeting CDKN2A/B it will provide a therapeutic way to treat neurofibromas. We have confirmed that there is an increase in CDKN2A/B mRNA and increase of INK4A/B protein level, as well as their downstream protein of P53, Rb in *Nf1*-deficient murine Schwann cells. The goal in this study is to evaluate the biological consequences of shRNA knockdown of CDKN2A/B to examine the impact of the CDKN2A tumor suppressor on Schwann cell proliferation, apoptosis and senescence. Furthermore, we will confirm that these changes in cell function are associated with a decrease in INK4a, p53, and pRB

Basic Science ***Research Technician***

POSTER #52

IN VIVO ANALYSIS OF NOVEL THERAPEUTICS TARGETING THE DNA DAMAGE RESPONSE

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The genetic alterations often observed in solid tumors impacting genome stability and maintenance, a hallmark of cancer, present the opportunity for therapeutic intervention via synthetic lethal interactions. We have targeted two proteins involved in the DNA damage response and repair: Xeroderma Pigmentosum Group A-complementing Protein (XPA) involved in nucleotide excision repair (NER) and Replication Protein A (RPA) involved replication, repair, and recombination. We report the preliminary analysis of three classes of small molecule therapeutics targeting these proteins. The XPA targeted compound 68, an X-80 derivative optimized for potency, solubility and metabolism, was administered to NSG mice in a PEG-400 formulation via intraperitoneal (IP) injections three times a week for one week. Compound was well tolerated up to 200mg/kg with no overt toxicity based on no loss of body weight and no organ abnormalities observed on necropsy. H&E staining of liver sections appeared normal. RPA targeted compound MCI administered IP in a DMSO/Tween-20 formulation was tolerated upon dose reduction to 50mg/kg while 551 was tolerated at the standard dose of 200mg/kg. Anti-tumor efficacy was observed in small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC) and pancreatic cancer xenograft models. More recent analyses towards optimization of the formulation have identified a corn oil excipient suitable for both IP and oral gavage dosing. In this formulation RPA compounds were well tolerated with no overt toxicity and a noticeable lack of the intraperitoneal organ abnormalities observed with previous formulations. Experiments using these optimized formulations will be carried out to determine efficacy alone and in combination with standard cisplatin therapies for the treatment of lung and ovarian cancer.

Basic Science ***Research Technician***

POSTER #53

TARGETING DNA-PK VIA SMALL MOLECULE INHIBITORS OF THE KU-DNA INTERACTION

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DNA-PK, the DNA-dependent protein kinase, is a validated target for cancer therapeutics that drives the DNA damage response and plays a critical role in the non-homologous end joining (NHEJ) DNA repair pathway. Inhibition of DNA-PK activity can elicit anticancer activity and also sensitize cancer cells to DNA damaging therapy. NHEJ is responsible for the repair of DNA double strand breaks (DSB), particularly those induced by ionizing radiation (IR). The generation of DNA DSBs is the mechanism of clinical efficacy of radiation therapy and numerous DNA damaging chemotherapeutic drugs used to treat various cancers. Modulating the pathway responsible for repairing these breaks has been shown to have a profound impact on the efficacy of IR or chemotherapy in the clinic. We have taken a completely unique and novel approach to inhibiting DNA-PK that is based on extensive knowledge regarding DNA-PK kinase activation and affords considerable advantages to current approaches in DNA-PK inhibition. DNA-PK activation and NHEJ pathway engagement requires binding of the Ku70/80 heterodimer to DNA ends and we are targeting this protein-DNA interaction. We have discovered and developed a series of highly potent and selective DNA-PK inhibitors that act via blocking the binding of Ku70/80 to DNA ends. The lead compound from this series of molecules inhibits DNA-PK catalytic activity at nanomolar concentrations, has single-agent activity in cancer cell lines, and potentiates cellular sensitivity to IR treatment and DSB-producing chemotherapeutic agents. These data demonstrate the utility of inhibiting DNA-PK via targeting the Ku-DNA interaction.

Basic Science ***Research Technician***

POSTER #54

INTRAOPERATIVE ASSESSMENT OF BREAST TUMOR MARGINS USING MULTIMODAL PHOTOACOUSTIC TOMOGRAPHY (MARGINPAT)

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Radical mastectomies are progressively becoming a surgery of the past. Women today are increasingly opting for lumpectomies, a less invasive treatment option. Clinical data has shown no difference in survival or clinical outcomes between the two surgical groups in early stages of breast cancer. There is however, an undesired outcome yet to be adequately addressed. Lumpectomies have, in some cases, failed to remove marginal malignant tumor cells left as a product from surgery.

Following surgery, tumor biopsies are analyzed for marginal tumor cells. Biopsies are cut in, fixed, processed in a tissue processor, embedded into a paraffin block, and stained with H&E. After processing, presence of marginal cancerous tissue is determined by a pathologist. This process takes approximately 3 days, ultimately requiring the patient to undergo reoperation if a positive margin is discovered. Presently, the reoperation rate is 20-45%.

A device capable of imaging removed tissue to determine remaining marginal tumor during surgery would greatly reduce the reoperation rate. Multiple intraoperative imaging tools existing or are emerging for breast tumor margin assessment. Current devices fail to meet acceptable clinical specifications due to either long procedure time (15 mins+), low sensitivity (~70%), and/or low specificity (~68%). An unmet need exists in developing an intraoperative margin assessment device that is rapid, sensitive, capable of measuring the entire tissue surface, and images a depth of 2mm+.

The MarginPAT device presents a multimodal photoacoustic/ultrasound imaging system for rapid and highly sensitive breast cancer margin assessment. After surgical removal of tumor, tissue is inserted via cartridge, into MarginPAT for imaging. An automatic scan (3 mm) is captured with high speed (20 cm^2 / min) and no exogenous labeling. These achieved specifications meet the needs of intraoperative margin assessment, which far surpass similar platforms. More importantly, MarginPAT inherently supports conventional ultrasound imaging, which is used in preoperation diagnosis and wire localization.

These multimodality functions allow MarginPAT to be utilized in multiple fields of breast cancer diseases, another advantage over other platforms. In conclusion, current clinical results show through both verification and validation that MarginPAT competently performs in the operating room.

Basic Science ***Senior Undergrad - MS1 at IU Next Year***

POSTER #55

A NOVEL GENETICALLY ENGINEERED MOUSE MODEL OF VULVAR CANCER

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Gynecological cancer remains a significant public health issue. In 2017, over 100,000 women will be diagnosed with a gynecologic cancer and more than 31,000 will die in the United States. Primary vulvar cancer accounts for less than 5% of gynecological cancers diagnosed, but the curative surgical treatment for deeply invasive tumors has considerable morbidity. Since vulvar cancer is an infrequent tumor, randomized trials of therapeutic approaches in women are uncommon. Thus, model systems are needed to understand the pathophysiology of the disease to develop improved therapies. To study female reproductive tract cancers, in particular endometrial cancer, we created a genetically engineered mouse model using the progesterone receptor-Cre (Pgr^{Cre}) which conditionally deletes in the uterus. When we deleted *Arid1a*, the proposed tumor suppressor that is thought to be a driver mutation in endometrial cancer, the female mice were infertile but did not develop endometrial cancer. Thus, we added oncogenic *Kras* ($Kras^{G12D}$). Surprisingly, these female mice ($Pgr^{Cre}Arid1a^{f/f}Kras^{SLS\text{-}G12D}$) developed large vulvar tumors with 100% penetrance by 8 weeks. Gross and histological examination of uterine and ovarian tissues revealed normal structures without malignancy or benign tumors. This mouse model may allow a better understanding of the molecular mechanisms underlying the genetic transformation of vulvar cancer and can be used to develop targets for therapy.

Basic Science ***Visiting Research Associate***

POSTER #56

UNDERSTANDING LUNG CANCER SCREENING BEHAVIOR: RACIAL, GENDER, AND GEOGRAPHIC DIFFERENCES AMONG INDIANA LONG-TERM SMOKERS

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Background: Lung cancer is the leading cause of all cancer-related deaths in the U.S. with more than 158,000 people dying annually; approximately 4,000 of those are Indiana residents. Disparities related to race, gender, and geographic area of residence exist in other types of cancer screening. However, little is known about how these key sociodemographic variables impact lung cancer screening behavior. Because stage at presentation drives mortality in lung cancer, screening high-risk smokers is critical for early detection. It is important to understand key factors that influence lung screening behavior and to examine factors that may explain disparities in order to intervene.

Purpose: The purposes of this study were to: 1) identify variables associated with lung screening behavior in screening-eligible individuals in the state of Indiana; and 2) examine potential differences in these key variables by race, gender, and geographic location.

Conceptual Framework: Conceptual Model for Lung Cancer Screening Participation

Methods: Cross-sectional, descriptive study using survey methodology with a purposive sample of 456 screening-eligible individuals from the state of Indiana using four primary recruitment methods.

Results: We recruited a diverse sample (265 females,191 males; 255 Whites,185 Blacks; and 251 residents from urban areas, 57 from suburban areas, and 148 from rural areas. Fifty-three (11.7%) participants reported they had a lung scan to screen for lung cancer in the past year while 401 (88.3%) participants had not. Differences between race (Black/White), gender (Female/Male), and geographic location (Rural/Suburban/Urban) were examined on health beliefs (perceived risk, perceived benefits, perceived barriers, self-efficacy) and key psychological variables (stigma, mistrust, fatalism, worry, fear) associated with lung screening. Significant differences were observed in all categories. Men had higher levels of mistrust compared to women ($p<0.0001$). White participants had higher lung cancer and screening knowledge scores ($p<0.0001$), self-efficacy for lung cancer screening scores ($p=0.0012$), and higher levels of perceived stigma ($p=0.0214$) compared to Black participants. Black participants reported higher perceived barriers to lung cancer screening scores ($p=0.0029$) and lung cancer worry ($p=0.0004$) compared to White participants. Suburban participants had the highest total lung cancer and screening knowledge scores ($p=0.0094$) compared to rural and urban residents. Rural participants perceived higher levels of stigma ($p=0.0134$) compared to urban and suburban participants, while urban residents had the highest perceived barriers to lung cancer screening ($p=0.0142$).

Conclusions: This is the first study to examine lung cancer beliefs by variables that may influence lung cancer disparities in screening. Results indicate there are key differences in individual health beliefs, perceived stigma, mistrust, and lung cancer worry that may perpetuate cancer screening behavior disparities. Barriers to lung cancer screening must be addressed as screening becomes more widely implemented, and it is important to consider health beliefs and key psychological variables as decision support tools are developed.

Behavioral ***Faculty***

POSTER #57

FINDING SOLUTIONS TO THE PSYCHOSOCIAL NEEDS OF PEDIATRIC CANCER PATIENTS AND FAMILIES: ONCOLOGY FRIENDS AND FAMILY (OFF) APP

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Mobile phone apps are increasingly being used in healthcare, and many have been developed for cancer care. However, almost none of them have addressed the psychosocial needs of patients and families with kids being diagnosed with cancer. The impact that friends and family members make to support each other during this time of need cannot be overemphasized, and is therefore proper to have a mobile app to help manage and organize the various activities and support that pediatric cancer patients, their parents, siblings, friends and other family members desire and go through for proper care coordination. Oncology Friends and Family (OFF) app was created to help address these psychosocial needs, as well as lay a good background for future developments in this area. We have developed an initial mockup of what is required to build an effective app that will cater to these needs for pediatric Leukemia patients and families, as well as provide features for these users to be able to reach out to existing friends and new users. They can reach cancer care associations for important needs such as questions about care, helping with sibling issues, finding and sharing success stories, as well as learning about their condition. Overall, we're building the app to help address options for finding physicians and specialists, care centers and caregivers, accessing laboratory results and care progress for patients through Health Information Exchanges, and communicating with authorized stakeholders via a secured messaging platform while providing quick search plus other features for users to ask for help, provide help, refer people to help, decline gracefully when unable to offer help and schedule or reschedule tasks as situation demands.

Behavioral ***Graduate Student***

POSTER #58

DEVELOPMENT AND PSYCHOMETRIC TESTING OF A NEW BODY IMAGE SCALE FOR BREAST CANCER SURVIVORS

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Purpose: The purpose of this study is to develop and psychometrically test a body image scale for breast cancer survivors. Psychometric analyses were guided by a theoretical framework that defines associations between body image and proximal (physical, psychological, and social) and distal (Index of Well-Being) quality of life outcomes.

Methods: Data for this study were obtained as part of a larger study that compared breast cancer survivors with acquaintance controls (women who were not diagnosed with breast cancer). Women (n=1127) aged 18-45 or 55-70 years at diagnosis, who were three to eight years from initial diagnosis, and who had received chemotherapy as part of their treatment were enrolled in a descriptive study designed to identify differences in breast cancer survivors diagnosed at a younger age versus those diagnosed at an older age. Cross-sectional data for this secondary analysis were collected via telephone and mailed questionnaires. Psychometric testing was conducted using item analysis, Cronbach's alpha, factor analysis, multiple hierarchical regression, and Pearson's correlations.

Results: Item analysis resulted in an eight item scale with Cronbach's alpha=0.88, inter-items correlations ranging from 0.135-0.783, and item-total correlation coefficients ranging from 0.367-0.829. One of the eight items was deleted because of redundancy identified by a high (>0.7) inter-item correlation. Factor analysis supported a unidimensional scale. Construct validity was demonstrated through multiple hierarchal regression with 45% of the body image variance explained by the constructs in the conceptual model [$F(14,784) = 48.35$, $p<0.000$]. Criterion validity, as tested with Pearson's correlations, supported the relationship of body image to both proximal and distal quality of life outcomes.

Conclusion: The body image scale demonstrated high internal consistency reliability, unidimensionality, and construct and criterion validity. The scale could be integrated into interventions targeting body image in breast cancer survivors and as an assessment tool for clinicians.

Behavioral ***Graduate Student***

POSTER #59

ADVANCED CANCER PATIENTS: DO WE REALLY KNOW THEIR SPIRITUAL NEEDS? A QUALITATIVE INQUIRY

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Problem: Advanced cancer patients face emotional and spiritual distress as they cope with their illness. Spirituality and religion (S/R) have been found to play a significant role in coping with the distress among cancer patients. Chaplains are the spiritual care experts in the health system, but have traditionally had a limited presence in the outpatient setting. As cancer care moves to this setting, understanding the S/R needs of advanced cancer patients in the outpatient setting is essential. This study was designed to assess the S/R needs of advance cancer patients with an estimated 12 months survival in outpatient care clinics.

Methods: We used across-sectional approach and the qualitative method of grounded theory in order to understand the S/R needs of advanced cancer patients. We specifically sought to understand S/R resources used by advanced cancer patients in coping with their illness. Using a semi-structured interview guide, interviews were conducted at the Indiana Simon Cancer Center outpatient clinic with patients whom their primary oncologist identified as having a <12 months estimated survival. The interviews were audio recorded by the primary investigator and transcribed. Two investigators who were blinded from interviewees as well as from each other's outcome assessment together with the primary investigator coded the transcribed data before using Nvivo for coding consistency. The codes from interrater findings were also analyzed and comparisons were made to ensure consistency.

Outcomes/Data: Four core needs emerged in this study. They include: longing for relationships "We are a lot closer. Can't go through this without but yeah, I would say we're a lot closer. We know a lot more about each other because we sit in here…and we have all kinds of conversations.", need to reconcile with God "I felt like I was going to go to hell if I didn't go to church… so that fell on me real heavy and so I decided to start going to church to become catholic." need to clarify emotional distresses,"At first I was kind of angry…I thought we made a covenant, God, and now You've just let me down" and need to identify coping resources. Understanding the definition of spirituality by advanced cancer patients and what that means to a patient may lead to better care of their spiritual needs. Ability to identify such spiritual needs and availability of spiritual resources that meet those needs together with professional delivery of those resources is a necessary prerequisite to successful coping. The understanding of what the S/R needs are for advanced cancer patients, the meaning of S/R, and their coping resources from this study may guide professional spiritual care providers to design a patient-based and outcome-oriented intervention that may reduce advanced cancer patients stress, improve communication, increase patient and staff satisfaction and cooperation.

Behavioral ***Graduate Student***

POSTER #60

THE GAMBLER: HOW OVARIAN CANCER PATIENTS USE METAPHORS IN ONLINE FORUMS TO DESCRIBE CLINICAL TRIAL EXPERIENCE

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Precision medicine through tailored drug therapies is a national initiative in the United States and priority at Indiana University for improving cancer treatments. This is particularly urgent for patients with cancers that have low 5-year survival rates, like ovarian. However, only 5 percent of individuals with cancer participate in clinical trials, the necessary precursor to drug approval. Extensive research focuses on identifying barriers to accrual and retention in trials to improve this percentage. However, few studies seek to examine the experience of individuals who are currently seeking access to or participating in experimental therapies to see if any insight can be gleaned from this population. The goal of this paper is to use metaphor analysis to develop a deeper understanding of the patient experience finding, accessing, and participating in clinical trials. It is possible that by making clinical trial communication and design more participant-centered, enrollment in them will increase. Metaphoric themes that emerge from forums include the following: finding the trial as a playing a difficult game or taking a difficult class; gaining access to the trial as gaining membership in an exclusive club; and participating in the clinical trial as a taking a gamble. With the tide of clinical research moving toward tailored drugs, understanding and attending to patient communication needs in clinical trials becomes pressing.

Behavioral ***Graduate Student***

POSTER #61

SYMPTOM CLUSTERS IN METASTATIC BREAST CANCER

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Co-occurring symptoms, or symptom clusters, have a negative, compounding effect on functional status and quality of life in cancer patients. Symptom clusters are also significant because they point to common mechanisms—both biological and psychological—that may underlie various symptoms. Psychoneurological symptom clusters consisting of mood disturbances, cognitive dysfunction, fatigue, sleep disturbance, and pain have been found in patients with early-stage breast cancer and other cancer types. Symptom cluster research in breast cancer patients has largely focused on patients with early-stage disease or survivors in remission, and little is known about symptom clusters in metastatic breast cancer patients despite their increasing longevity and high symptom burden. In order to address this gap, this study aimed to identify physical and psychological symptom clusters in metastatic breast cancer patients. Eighty women with metastatic breast cancer (91% Caucasian, average age = 55 years, SD = 11 years) were recruited from the Indiana University Simon Cancer Center to participate in this cross-sectional telephone interview study. The interview included valid measures of the severity of 10 common symptoms (i.e., pain, fatigue, anxiety, depressive symptoms, neuropathy, edema of arms or legs, nausea, hot flashes, sleep disturbance, and cognitive concerns). An exploratory cluster analysis was performed on measures of all 10 symptoms. Anxiety, depressive symptoms, fatigue, sleep disturbance, and cognitive concerns were found to cluster. A separate cluster consisted of pain, neuropathy, and nausea. Hot flashes and swelling did not cluster. The first cluster is consistent with a psychoneurological symptom cluster, which has been found in patients with non-metastatic breast cancer and other cancer types. The co-occurrence of pain and neuropathy may reflect neuropathic pain, which, along with nausea, is a common side effect of chemotherapy. Further research is needed to replicate these clusters and elucidate common mechanisms underlying co-occurring symptoms to inform intervention development.

Behavioral ***Graduate Student***

POSTER #62

COMMUNICATION FACTORS AT TIME OF PEDIATRIC CANCER DIAGNOSIS: A SYSTEMATIC REVIEW

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The cancer diagnosis of a child is emotionally difficult on caregivers and the way it is communicated can affect the relationship with the medical system. There are limited data on communication factors caregivers find important at time of their child's cancer diagnosis. A comprehensive evaluation of such factors could improve clinical care and patient-centered outcomes.

The objective of this study is to systematically examine the caregiver's perspective regarding important elements of communication that medical providers can follow at the time of delivery of the cancer diagnosis.

We searched Ovid, EMBASE, Scopus, World of Science and EBSCO databases for prospective or retrospective qualitative or quantitative studies related to communication between caregivers and medical providers regarding children with new cancer diagnoses. Two independent researchers performed title, abstract and full text review. Four main themes emerged from the analysis of communication factors identified by caregivers: Provider Communication Style, Delivery Content, Logistics and System Issues.

15 studies were included in this qualitative systematic review including 13 retrospective and 2 prospective studies. Most US studies used quantitative methods, whereas non-US studies used qualitative or mixed methods methods. Elements that caregivers consistently found important in a conversation around their child's diagnosis were a private, quiet location, with a provider delivering a clear message that allowed for hope. Caregivers found attentiveness and sensitivity to be two important characteristics under the Provider Communication Style category. In multiple studies, caregivers emphasized the importance of honesty (n=3), as well as a better understanding of possible alternatives to treatment (n=4). Finally, systems-level issues were highlighted as caregivers expressed frustration with conflicting messages from multiple providers and poor continuity of provider care.

Our results from this systematic review suggest that caregivers have a common set of expectations from their providers at the time of the delivery of their child's cancer diagnosis. This set of suggestions could be used to form practical guidelines for pediatric oncology practitioners (MDs, NPs, RNs) to use when delivering diagnostic information to new families.

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

POSTER #63

BIOLOGICAL EFFECTS ON BREAST CANCER SURVIVORS AFTER PSYCHOLOGICAL INTERVENTION

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Telomeres exist on the ends of chromosomes to protect and maintain their function. It is known that telomeres naturally shorten with age. In recent years, a strong link between life stress and telomere shortening has been drawn that implicates the 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 hypothesize that psychological condition could be associated with telomeres and mtDNA levels, contributing to susceptibility of cancer. Most cancer survivors experience fear of cancer recurrence (FCR), of which half report clinically significant levels. Thus, the objective of this study is to investigate whether placing breast cancer survivors into a certain intervention therapy group will result in a change of their telomere length and/or mtDNA levels. Seventy-six breast cancer survivors were split into three groups: those receiving six weeks of Acceptance and Commitment Therapy (ACT), those receiving six weeks of Survivorship Education workshops (SE), and those undergoing usual post-cancer care (UC). Telomere lengths and mtDNA levels were measured at these time-points: baseline (T1), post-intervention (T2), 1-month follow-up (T3), and 6-month follow-up (T4). The preliminary results show that women receiving ACT and SE have lost less telomere length than those undergoing usual care, indicating that the therapies show a positive impact on telomere length. We propose that accelerated cellular aging associated with FCR may be reversed further once survivors begin to cope more effectively using the techniques they learned during the interventions.

Behavioral ***undergrad /part-time tech***

POSTER #64

NO ASSOCIATION BETWEEN VITAMIN K INTAKE AND PROSTATE CANCER RISK IN THE PROSTATE, LUNG, COLORECTAL, AND OVARIAN CANCER SCREENING TRIAL (PLCO)

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Prostate cancer is one of the most common cancers worldwide. The etiology of prostate cancer largely remains unclear, with almost all established risk factors (e.g. age, race, family history) being not modifiable. Ecological, migrant, and temporal trend studies suggest that diet plays role in the occurrence of prostate cancer, but few nutrients that alter its risk have been identified from case-control and cohort studies. Vitamin K has two forms that naturally occur in foods, i.e., phylloquinone (vitamin K1) and menaquinone (vitamin K2). While phylloquinone is abundant in green leafy vegetables and some vegetable oils, menaquinone is primarily derived from fermented food products (e.g. cheese). Although experimental studies have shown that vitamin K inhibits the growth of various cancer cell lines (including prostate cancer cells), only one epidemiologic study has investigated the association between vitamin K intake and prostate cancer risk. In that German study, a significant inverse association with advanced prostate cancer was observed for menaquinone intake. The present study thus sought to investigate the associations between intake of vitamin K (from both dietary and supplemental sources) and the risk of total and advanced prostate cancer among 28,356 PLCO participants. A total of 3,097 cases of prostate cancer (including 511 advanced cases) have been documented during a median follow-up time of 11.6 years. Advanced prostate cancer were defined as disease in stage II with Gleason Score of 8-10, stage III, or stage IV. Usual dietary intake among study subjects was assessed with Dietary Questionnaire (DQX) at baseline and Dietary History Questionnaire (DHQ) at year 3, both of which were developed and validated by the National Cancer Institute. Dietary intake of phylloquinone and menaquinones were calculated using the USDA National Nutrient Database for Standard Reference (Release 23), supplemented with menaquinone data from published studies. Cox proportional hazards regression was performed to estimate hazard ratios (HRs) and 95% Confidence intervals (CIs) for dietary intake of phylloquinone and menaquinones in relation to prostate cancer risk. After adjustment for confounders, no statistically significant were found between vitamin K intake estimated from the DQX and prostate cancer risk [HR (95% CI)] for the highest quintile vs. the lowest quintile: 0.97 (0.83, 1.13) for total vitamin K, 0.97 (0.83, 1.13) for phylloquinone, and 1.00 (0.86, 1.17) for total menaquinones]. Overall null results were also observed when additional analysis was carried out by the stage of the disease (i.e. advanced and non-advanced cases) and for vitamin K intake data assessed with the DHQ. In summary, the present study revealed that intake of total vitamin K and its two natural forms was not associated with the risk of total and advanced prostate cancer.

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

POSTER #65

ANALYSIS OF ONE-CARBON NUTRIENTS AND THE DEVELOPMENT OF PANCREATIC CANCER

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Pancreatic cancer is one of the most fatal cancers, with a 5-year survival rate of only 8.2% in the United States. The etiology of pancreatic cancer largely remains elusive, and cigarette smoking is the only established risk factor. To prevent this disease, it is critical to identify its modifiable risk factors. One-carbon nutrients, such as folate, vitamin B_6, vitamin B_{12}, and methionine, are involved in DNA methylation and synthesis. Therefore, it is biologically plausible that these nutrients are associated with the risk of pancreatic cancer. However, epidemiologic evidence on this association is sparse and inconsistent. The present study was thus conducted to investigate the associations of intake of folate, vitamin B_6, vitamin B_{12}, and methionine with the risk of pancreatic cancer in a population-based case-control study conducted in 1994-1998. Cases (n=150) were recruited from the Mayo Clinic and all hospitals in the metropolitan areas of the Twin Cities, Minnesota. Controls (n=459) were randomly selected from the Twin Cities' seven-county metropolitan communities and were frequency matched by sex and age (within 5 years). Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (95% CI) for pancreatic cancer risk. After adjustment for confounders, dietary intake of folate was associated with a reduced risk of pancreatic cancer [quartile (Q) 4 vs. Q1: OR = 0.31, 95% CI = 0.12-0.78]. Inverse association was also observed for dietary intake of vitamin B_6 (Q4 vs. Q1: OR = 0.47, 95% CI = 0.19-1.17). No significant associations were seen for intakes of vitamin B_{12} and methionine. In conclusion, the present study suggests that high intake of folate and vitamin B_6 may confer some protection against the occurrence of pancreatic cancer.

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

POSTER #66

THE IMPACT OF EDUCATIONAL LEVEL ON MORTALITY: A POOLED ANALYSIS OF OVER 646,000 INDIVIDUALS IN THE ASIA COHORT CONSORTIUM

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Background: Most studies that have evaluated the association between educational level and the risk of death have been conducted in the United States and Europe. A limited number of studies have assessed this relationship in Asian populations.

Method: We performed a pooled analysis to evaluate the association between educational level and the risk of death among 646,495 individuals from 14 prospective cohort studies in the Asia Cohort Consortium. The analysis included 75,867 deaths that occurred during a mean follow-up period of 11.6 years, among which 26,073 were cancer-specific deaths and 24,674 were cardiovascular disease (CVD)-specific deaths. We employed cox proportional hazards regression models to estimate hazard ratios (HRs) and 95% confidence intervals (95% CIs) for the association between educational level and the risk of death.

Results: Among all combined cohorts, higher educational level was significantly associated with lower risk of death from all causes; compared to = primary school, HR and 95% CI for secondary education, trade/technical education, and = university degree was 0.87 (0.86-0.89), 0.78 (0.76-0.81), and 0.64 (0.62-0.66), respectively (Ptrend <0.0001). Compared with low educational level (i.e., = primary school), higher educational level was also significantly associated with lower cancer-specific mortality [HR (95% CI) for secondary education = 0.92 (0.89-0.95); for trade/technical education = 0.85 (0.81-0.89); and for = university degree= 0.73 (0.69-0.77); Ptrend <0.0001], as well as lower CVD-specific mortality [HR (95% CI) for secondary education= 0.86 (0.83-0.89); for trade/technical education = 0.76 (0.73-0.80); and for = university degree= 0.61 (0.58-0.65); Ptrend <0.0001]. The association appeared similar pattern between East Asians and South Asians; regarding all-cause mortality, compared to = primary school, HR and 95% CI for = university degree was 0.64 (0.62-0.67) (Ptrend <0.0001) among 491,785 East Asians (Chinese, Japanese, and Korean) and 0.71 (0.65-0.77) among 154,710 South Asians (Indians and Bangladeshis).

Conclusion: Higher educational level was associated with substantially reduced risk of death in Asian populations including both East and South Asians.

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

POSTER #67

STATIN USE AND NON-MELANOMA SKIN CANCER RISK: A META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS AND OBSERVATIONAL STUDIES

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Background: Existing evidence of the association between statin use and non-melanoma skin cancer (NMSC) risk has been inconsistent.

Methods: Meta-analysis of all eligible prospective observational studies and randomized controlled trials using a random-effects model

Results: Our meta-analysis of 14 randomized controlled trials (RCTs) including 63,157 subjects and 1,211 NMSC events showed no significant association between statin use and NMSC risk (RR=1.09, 95%CI=0.85-1.39; P for heterogeneity= 0.02). However, meta-analysis of four observational studies including 1,528,215 participants and 55,793 NMSC cases showed significantly increased risk of NMSC among statin users compared to non-users (RR=1.11, 95%CI=1.02-1.22; P for heterogeneity= 0.002). Furthermore, ever using lipophilic statins(RR=1.14, 95%CI=1.04-1.24) or lower-potency statins(RR=1.14, 95%CI=1.03-1.26), as well as usage of any statin longer than one year (RR=1.14, 95%CI=1.09-1.18)were significantly associated with increased NMSC risk based on observational studies.

Conclusions: Evidence from prospective observational studies supported an association between statin use and increased NMSC risk. This finding should be interpreted with caution due to modest number of included studies, possible between-study heterogeneity and inherent limitations of observational studies.

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

POSTER #68

COMPARISON OF CHANGES IN WAIST CIRCUMFERENCE AND BODY MASS INDEX (BMI) THROUGH ADULTHOOD AND THEIR RELATIONSHIP WITH ADVANCED COLORECTAL NEOPLASIA

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Background: Waist circumference (WC) is a stronger predictor of colon cancer risk than body mass index (BMI); however, how well change in either WC or BMI over time predicts the risk of AN remains unclear.
Purpose: To investigate 1) the relationship between change in adiposity measures (BMI and WC) from early adulthood to older age and the risk of advanced colorectal neoplasia (AN) and 2) whether WC change or BMI change is a stronger predictor of risk of AN.
Methods: The sample included 4,500 adults, 50-80 years with no previous colorectal cancer or adenomatous polyps and undergoing first-time screening colonoscopy. Participants reported adiposity measures (height, weight and WC) at early adulthood (age 21) and at time of screening (mean age 57). Changes in BMI were categorized as 1) increase from healthy BMI to overweight or obese, 2) increase from overweight to obese, and 3) stable BMI level at age 21 and time of screening. WC was defined as high-risk for females with a WC =35 and males =40 inches. Changes for WC were categorized as 1) increase from a low-risk WC to a high-risk WC, and 2) stable-risk WC at age 21 and time of screening. Gender, age, family history, exercise, aspirin use, alcohol, smoking and intake of red meat and vegetables were included in the logistic models.
Results: Being obese both at age 21 and at screening resulted in a significant increased risk of AN (OR=1.87; 95% CI 1.08-3.23) compared to those with a stable-healthy BMI at both time points. Stable overweight BMI at age 21 and at screening (OR=1.54; 95% CI 0.97-2.45) and increased BMI from healthy to overweight or obese and overweight to obese were not associated with AN, as compared to those who had a healthy BMI at both time points. Compared to those with a low-risk WC at both time points, those who had a high-risk WC at age 21 and at time of screening had a two-fold increased risk of AN (OR=2.15; 95% CI 1.35-3.45). Increasing WC from age 21 to time of screening compared to those with low-risk WC was not associated with risk of AN (OR=1.23; 95% CI 0.97-1.57).
When both WC and BMI change were included, WC change (=10.15, 2 DF, *p-value*=0.0062) but not BMI change (=5.66, 2 DF, *p-value*=0.34) predicted risk of AN.
Conclusions: Maintaining a high-risk WC or obese BMI is associated with increased risk for AN; however, when BMI is controlled, maintaining a high-risk WC may independently increase the risk for AN. Practitioners should caution adults with high-risk WC and obese BMI values to better control their adiposity.

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

POSTER #69

ASSESSMENT OF EFFICACY OUTCOMES IN TREATMENT OF HEPATIC METASTASIS TREATED WITH STEREOTACTIC BODY RADIATION THERAPY IN OLIGOMETASTATIC PATIENTS WITH PRIMARY COLON AND RECTAL CANCER

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Purpose

The purpose of this single-institution retrospective study was to assess clinical outcomes following stereotactic body radiation therapy (SBRT) including local control, progression-free survival (PFS), overall survival, and extra- and intra-hepatic progression-free survival in patients with liver metastases due to colorectal cancer. We compared our outcomes to the treatment arm of a recently published Phase III trial of FOLFOX chemotherapy with or without yttrium-90 microspheres, which demonstrated increased hepatic PFS in patients who received liver directed therapy plus chemo.

Patients and Methods

Eligible patients had liver metastases due to colorectal cancer, were treated with SBRT at Indiana University Simon Cancer Center between 2007-2015, and had at least 3 months of follow up. We identified 48 patients that met these criteria. The majority of patients had a solitary liver metastasis (81.6%); 9 patients (18.4%) had multiple metastases, and 2 of these had extrahepatic disease. The maximum number of treated liver metastases was 3. Median dose was 54 Gy (range 32-60 Gy) in 3 fractions (range 3-5). The mean tumor diameter was 2.8 cm (range 0.4-5.6). 81% of patients had received chemotherapy prior to SBRT. The Kaplan-Meier method was used to estimate actuarial survival times.

Results

At a median follow up of 29 months (range 3-75 months), crude local control was 91.7% (45/48 treated patients). Median overall survival was 43 months (95% CI, 31.3-54.7), and median progression-free survival was 16.0 months (95% CI 2.6-29.4). Median intrahepatic progression-free survival was 26 months (95% CI 12.2-39.8), longer than the estimated median extrahepatic progression-free survival of 23 months (95% CI 12.4-33.6). The crude overall rate of hepatic progression was 39.5% (19/48 treated patients), while the crude rate of extrahepatic progression was 45.8% (22/48 treated patients)

Conclusions

SBRT is very effective in the management of hepatic oligometastases and was associated with an excellent local control rate of 91% in this series. Outcomes with SBRT in our series compared favorably to Y90 treatment in the Phase III SIRFLOX study. Further research is needed to determine the optimum sequencing and combination of liver SBRT with cytotoxic chemotherapy and novel approaches such as immunotherapy.

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

POSTER #70

EFFECT OF ALCOHOL USE AND SMOKING ON GENE EXPRESSION IN NORMAL BREAST TISSUE

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Introduction: Alcohol use has been associated with increased risk of breast cancer, more consistently among heavy drinkers than moderate drinkers. While the association of smoking remains mixed, increasing evidence suggests smoking is linked to a higher risk of breast cancer in younger, premenopausal women. Additionally, alcohol and smoking are correlated and are known confounders of each other in relation to breast cancer. We hypothesized that alcohol use and smoking act on gene expression through which they influence breast cancer risk. Therefore, we conducted a gene expression study to explore the biological mechanisms underlying the association between alcohol/tobacco and breast cancer.

Methods: To examine the association of alcohol use and smoking with gene expression, we analyzed RNA-seq data obtained from normal breast tissue samples from 22 healthy women (K), and 37 tumor tissue (T) and 18 matched adjacent normal (AN) breast tissue samples from breast cancer patients. We performed generalized linear regression analysis adjusting for sequencing batch, age, and race, further adjusting for income and age at menarche in the K samples. Both independent and joint effects of alcohol use and smoking were evaluated by cross tabulation to identify differentially expressed genes, which were further examined for their biological significance. Breast cancer subtype analyses were also performed based on estrogen receptor (ER) status.

Results: In general, we observed that the number of differentially expressed genes was greatest in AN samples, followed by T and K samples. Alcohol use independently influenced the expression of genes related to glucose homeostasis (T: *CARTPT*, AN: *ERO1-LB*) and innate immunity (T: *MAGT1*, AN: *RC3H2*) in T and AN samples. We didn't observe significant change in gene expression associated with smoking in the T samples, but the expression of genes/transcripts related to fatty Acyl-CoA synthesis or lipid-lipoprotein metabolism (K: *PANK2*, *SLCO1A2*, AN: *PIK4K2A*, *HACD3*) were highly associated with smoking in K and AN samples. Lastly, the joint effect of both alcohol and smoking revealed novel genes related to lipid, glucose or protein metabolisms and both innate and adaptive immunity in T and AN samples (T: *PAQR1*, *SPINK5*, AN: *HCF-2*, *MED20*), in addition to genes identified in the independent association. Although associations also differed by ER status we were limited by power in the subtype analysis.

Conclusions: For both alcohol use and smoking, differential gene expression was largely observed in the adjacent normal samples compared to tumor and normal tissue samples. We did not observe significant change in gene expression influenced by smoking in tumor samples. Joint analysis revealed additional set of genes whose expression was influenced by both alcohol use and smoking, suggesting that the two risk factors may interact with each other, either enhancing or reducing their individual effects. Further research is needed to tease apart these effects and determine if any of these genes and biological mechanisms show consistent associations with breast cancer.

POSTER #71

PHOSPHODIESTERASE TYPE 5 INHIBITORS AND RISK OF MELANOMA: A META-ANALYSIS

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Background: Current evidence from individual observational studies about the association between phosphodiesterase type 5 (PDE5) inhibitors and risk of melanoma is controversial. We aimed to quantify the possible association between use of PDE5 inhibitors and risk of melanoma by meta-analysis of current evidence.

Methods: Pubmed, Embase, CENTRAL, Web of Science, and ClinicalTrial.gov were searched up to July 13, 2016 to identify the randomized trials and cohort or case-control studies that reported the outcomes of skin cancer associated with the use of PDE5 inhibitors. The primary outcome of interest was melanoma and the secondary outcomes including basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Two reviewers independently selected studies, extracted data, and assessed the quality of studies. Random effects meta-analyses were used to calculate the pooled odds ratio (OR) with 95% confidence interval (CI).

Results: Five observational studies were included. Compared with PDE5 inhibitor non-use, PDE5 inhibitor use was slightly but significantly associated with increased risk of melanoma (OR 1.12, 95% CI 1.03-1.21, I^2= 49.1%)) and BCC (OR 1.14, 95% CI 1.09-1.19, I^2= 49.5%), but not SCC (OR 1.04, 95% CI 0.78-1.37, I^2 = 16.9%). For melanoma risk, none of the pre-specified factors (dose of PDE5 inhibitors, study design, and study region) significantly affected the results (P>0.05). Our sensitivity analysis confirmed the stability of the results.

Conclusions: Use of PDE5 inhibitors may be associated with a slightly increased risk of melanoma and BCC, but not SCC. However, further large well-conducted prospective studies with adequate adjustment for potential confounders are required for confirmation.

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

POSTER #72

USE OF ANTIHYPERTENSIVE DRUGS AND RISK OF SKIN CANCER: A META-ANALYSIS OF OBSERVATIONAL STUDIES

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Background: Several antihypertensive drugs are photosensitizing and may promote the development of skin cancer.However, existing epidemiological evidence for an association between use of antihypertensive drugs and risk ofskin cancer is inconsistent. We aimed to quantify the association between antihypertensive drugs and skin cancer.

Methods: Pubmed, Embase, and CENTRAL from inception toAugust 12, 2016 and references of relevant articles to identify observations studies that evaluated the association between use of antihypertensive drugs and risk of skin cancer. A random-effects meta-analysis was used to estimate the adjusted odds ratio (OR) with 95% confidence interval (CI).

Results: For melanoma (MM) risk, we included eight observational. Compared with non-use, use of diuretics (OR, 1.10; 95% CI, 1.03 to 1.17) or beta-adrenergic blocking agents (ß-blockers) (OR, 1.19; 95% CI, 1.04 to 1.37) was significantly associated with increased risk of MM. The use of angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), and calcium channel blockers (CCBs) was positively associated with increased risk of MM, but not statistically significant. For non-melanoma skin cancer (NMSC) risk, consisting of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), ten observational studies were included. Compared with non-use, diuretic use was significantly associated with increased risk of BCC (adjusted OR, 1.10; 95% CI, 1.01 to 1.20) and SCC (adjusted OR, 1.40; 95% CI, 1.19 to 1.66). ß-blockers and CCBs were slightly associated with increased risk of BCC (but not SCC) with an adjusted OR of 1.09 (95% CI, 1.04 to 1.15) and 1.15 (95% CI, 1.09 to 1.21), respectively. ACE inhibitors and ARBs were not associated with increased risk of NMSC.

Conclusions: Use of diuretics was slightly but significantly associated with increased risk of skin cancer. The risk of skin cancer associated with other antihypertensive drugs (ACE inhibitors, ARBs, ß-blockers, and CCBs) was unclear. Our findings suggest close monitoring of skin cancer risk in patients under hypertensive management (especially use of diuretics) and stimulate further investigation to clarify the possible mechanism.

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

POSTER #73

MOLECULAR ALTERATIONS IN THE BREAST ASSOCIATED WITH EARLY MENARCHE

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Background: Menarche, the onset of the female menstruation, is a marker of pubertal timing. Age at menarche varies widely between girls and is highly dependent on nutritional status and body fat accumulation. The occurrence of menarche at an early age is linked to an increased risk of several adverse health conditions later in life, such as obesity, type-2 diabetes, breast and endometrial cancer, and cardiovascular disease. Indeed, for every one year decrease in age at menarche (from an average age at menarche of 12.5 years), breast cancer risk is increased by 5%. Several genome wide association studies (GWAS) have identified genetic variants (i.e. in the *LIN28B* gene) that are associated with early age at menarche, however little is known about the changes occurring in the breast tissue of women with early menarche. We hypothesize that early age at menarche results in permanent molecular alterations in the breast tissue and that those abnormalities may contribute to the tissue's susceptibility to carcinogens and breast cancer development.

Methods: To test our hypothesis we used the resources available at the Susan G. Komen Tissue Bank at the IU Simon Cancer Center (KTB). We selected histologically normal breast tissue from healthy, young women with either early (age = 10 years) or late menarche (age = 15 years), and matched for age, race, BMI, and menstrual phase. Breast tissue biopsies from these women were microdissected to isolate the breast epithelium and next generation RNA-sequencing was used to generate a transcriptome profile for each sample. Differential expression was performed using DESeq2 in R. The stroma of each of the samples was also evaluated using immunostaining.

Results/Conclusions: Preliminary data show significant differences when comparing the transcriptome profiles of the microdissected breast epithelium from the early and late menarche sample cohorts. The tissue from women with early menarche had upregulation of genes associated with defense against oxidative stress and/or infectious bacteria (lactotransferrin (LTF), ceruloplasmin (CP)), cell adhesion, (ITGa11, ITGaX, ITGaM, ITGaL, ITGb2), immune response (CARD9, LAIR1), and had downregulation of ubiquitination pathways (USP40, AMFR) and lipoprotein metabolism (OSBPL1A, LIPH, PIGN). Markers of oxidative stress (LTF, CP), cell proliferation (Ki67), and immune infiltrates (CD45, CD20, CD8, CD68) using immunostaining is underway. Together, this information will give us the opportunity to better understand early age at menarche as a breast cancer risk factor and advance research for women's health.

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

POSTER #74

CLUSTERING OF PIGMENTATION RISK FACTORS FOR NON-MELANOMA SKIN CANCER AMONG INDIANA RESIDENTS

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Introduction: Skin cancer is the most common type of cancer in the United States. One in five Americans will develop skin cancer in their lifetime. Three major types of skin cancer are basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma. While SCC and BCC are most common forms, melanoma is the most fatal. Pigmentation traits such as skin color, eye color, and hair color are known risk factors for all three types of skin cancer. A person with fair skin, light-colored eyes, red or blond has an increased risk of skin cancer.

Methods: We evaluated characteristics of 84 non-melanoma skin cancer patients enrolled from Department of Dermatology at Indiana University Hospital. Using self-reported questionnaire data on baseline health-related exposures and skin cancer- related factors, we examined the distribution of known skin cancer risk factors in these BCC and SCC patients. We further evaluated the clustering of pigmentation risk factors in the patients including skin color, eye color, and hair color.

Results: As expected, we observed more male patients in both BCC and SCC (74% in each). When assessing pigmentation risk factors individually, we found that BCC patients tend to have more fair skin (52%), blue/light-colored eyes (52%), and read/blonde hair (44%). These percentages are 65%, 35%, and 27%, respectively, in SCC patients. SCC patients tend to have more fair skin (65%), hazel/green/medium-colored eyes (41%), and dark brown hair (35%), and these percentages are 52%, 28%, and 28%, respectively, in BCC patients. When examining the clustering of the three pigmentation risk factors, we observed the following patterns: In BCC patients, the most common combination is “Fair skin, Blue/Light-colored eyes, and Red/Blonde hair” (26%), followed by “Medium-colored skin, Brown/Dark-colored eyes, and Dark Brown/Black” (16%) and “Fair skin, Hazel/Green/medium-colored eyes, and Red/Blonde hair” (12%). In SCC patients, the most common combinations are “Fair skin, Hazel/Green/medium-colored eyes, and Dark Brown/Black hair” (15%) and “Medium-colored skin, Blue/Light-colored eyes, and Light Brown hair” (15%), followed by “Fair skin, Hazel/Green/medium-colored eyes, and Red/Blonde hair” (12%) and “Fair skin, Brown/Dark eyes, and Dark Brown/Black hair” (12%).

Conclusions: Patients tend to have more fair skin in both BCC and SCC patients. However, eye color and hair color showed less consistent patterns in BCC and SCC patients. The clustering of the three pigmentation risk factors appeared to be different in BCC and SCC patients, suggesting different underlying molecular mechanisms and/or interactions of these traits for distinct types of non-melanoma skin cancer. Further research is needed to replicate the findings in larger study and understand the etiology of different types of skin cancer.

Population Science/Epidemiology ***high school student mentored by faculty member***

POSTER #75

REDUCED CIRCULATING MAIT CELLS IN OBESITY

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Obesity is a major risk factor for certain types of cancer. The mechanisms by which obesity contributes to tumor metastasis are not well understood. The immune system is known to be essential in fighting against tumors, and obesity causes systemic chronic inflammation with altered immune responses. Recent studies have revealed that gut microbiota also contributes to obesity and the pathology associated with obesity. Mucosal-associated invariant T (MAIT) cells are a unique subpopulation of T cells that are characterized by the expression of semi-invariant TCR (Va19 in mice; Va7.2 in humans). Their development and maturation require the gut microbiota and antigen presenting molecule MR1. The function of MAIT cells is currently not well studied. We have found reduced circulating MAIT cells in obese patients compared to age- and gender-matched healthy donors. We also used real-time PCR to determine the expression levels of the invariant TCR of MAIT cells and transcription factors of conventional T cells. We found, although no difference was observed in any gene expression analyzed between samples from obese patients or healthy donors, mRNA expression of TCR Va7.2 positively correlated with transcription factor TBX21 (for T helper 1 cells) and BCL6 (for T follicular helper cells). Our work suggests that obesity alters MAIT cell function. The correlation of MAIT cell TCR gene expression with transcription factors of other effector T cells suggests there may be transcriptional pathways linking MAIT cells to the function of conventional T cells. Future work will focus on determining how obesity impacts the function of MAIT cells.

Population Science/Epidemiology

POSTER #76

GENOMIC AND EPIGENOMIC SIGNATURES OF PLATINUM RE-SENSITIZATION IN OVARIAN CANCER

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Epigenetic changes, particularly DNA methylation aberrations have been implicated in acquired resistance to platinum in ovarian cancer. A multi-institutional randomized clinical trial compared a regimen of a DNA methyl transferase (DNMT) inhibitor guadecitabine and carboplatin to physician's choice chemotherapy for patients with recurrent platinum resistant ovarian cancer. Tumor biopsies or malignant ascites were collected at baseline and after two cycles of treatment. The goal of the current study was to analyze guadecitabine-induced DNA methylation and gene expression changes in relation to clinical outcomes. Genomic and epigenomic profiling using the Infinium HumanMethylation450 BeadChip (HM450) and RNA sequencing revealed extensive methylation and gene expression changes induced by guadecitabine in ovarian tumors. 94 gene promoters were significantly hypomethylated after treatment with guadecitabine and 949 genes were differentially expressed in pre vs. post-treatment tumors. Pathways associated with immune reactivation and DNA repair were significantly altered by guadecitabine treatment. Expression levels changes affecting 1155 genes involved in 23 networks correlated with progression free survival. Increased expression of selected genes (e.g. *DOK2, miR193a*) silenced through promoter methylation restored platinum sensitivity in ovarian cancer cells. Together, these results support that guadecitabine altered DNA methylation and expression of genes and gene networks correlates with re-sensitization to carboplatin in patients with heavily pre-treated ovarian cancer.

Translational/Clinical Research ***Faculty***

POSTER #77

PHOSPHOPEPTIDE MAPPING OF DLC1 IN ER+ BREAST CANCER REVEALS AMOTL2, A KEY HIPPO PATHWAY COMPONENT, AS AN IMPORTANT TARGET

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Background: Deleted in Liver Cancer-1 (*DLC1*), a Rho-GTPase-activating protein (GAP), functions as a tumor suppressor gene in a number of cancers and regulates cancer cell growth and progression. However, its role and clinical relevance is unclear in breast cancer. It is essential to understand the impact of *DLC1* and its functional network in preventing tumor progression in breast cancer.

Design: Expression of *DLC1* was correlated with prognosis using publicly available breast cancer gene expression data sets and quantitative Reverse Transcription PCR (qRT-PCR) in cohorts of ER+ breast cancer. Overexpression of full-length *DLC1* (*DLC1-FL*) expression in T47D cells was generated using mammalian expression cloning vector, pcDNA3.1+/C-(K)-DYK, and CloneEZ™ technology. Growth rate of control and *DLC1-FL* knock-in cells was assessed for 2 weeks using clonogenic assay. Proteomic and phosphopeptide enrichment assays (Pierce TiO2 enrichment kit) were performed in triplicates to examine the basis of altered growth phenotype. The PeakJuggler node in Proteome Discoverer was utilized for label-free quantitation of both protein and peptide MS peak areas. In addition, GO term enrichment analysis was performed in DAVID for the significantly changed phosphopeptides.

Results: Low expression of *DLC1* correlates with poor prognosis in patients with ER+ breast cancer. Stable knock-in of T47D-DLC1-FL inhibits cell growth significantly in vitro compared to T47D-control in clonogenic assay. The phosphopeptide enrichment proteomic analyses showed 199 phosphopeptides were identified only in T47D-DLC1-FL and 182 peptides were identified only in T47D-control cells. Pathway analysis using DAVID showed three main clusters of differentially identified phosphopeptides (p-values = 0.01) involving cadherin binding (p=4.9e-12), cell-cell adherens junction (p=3.9e-10), and cell-cell adhesion (p=9.4e-7). Analysis of specific phosphopeptides showed canonical pathways such as CTNNB1 (Thr552), and BCL2L13 (370-385). More importantly, phosphorylation of HIPPO-pathway component AMOTL2 at S766, critical for promoting YAP signaling and invasion was only identified in T47D-control but not T47D-DLC1-FL (p=5.78e-5).

Conclusions: This data suggest that the absence of DLC1 promotes phosphorylation of AMOTL2, and thereby activating YAP-TAZ signaling leading to invasiveness. This data provides mechanistic basis for targeting this pathway to prevent recurrence in ER+ breast cancer.

Translational/Clinical Research ***Faculty***

POSTER #78

POSTOPERATIVE RADIATION FOR TUMOR CONTROL AND OVERALL SURVIVAL IN THYMIC EPITHELIAL TUMORS (TET): A MATCHED-PAIR ANALYSIS

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Background:

Due to lack of randomized trials, the role of postoperative radiation therapy (PORT) in thymic epithelial tumors (TET) remains controversial. This study aimed to evaluate whether PORT improves tumor control and overall survival (OS) in patients with resected TET in a large single institution database.

Methods:

This is a retrospective study of all TETs seen at Indiana University between 1975 and 2016. Patients with resected thymoma (T) or thymic carcinoma (TC) were eligible disregarding their margin status or stage. Study endpoints were progression free survival (PFS) and OS. Age, gender, race, tumor size, stage, pathology, grade, completeness of resection and adjuvant treatment modality were analyzed for significance on PFS and OS. Multivariate Cox model was used to identify significant factors for propensity score matching. Differences between the PORT and surgery alone group were estimated using stratified log-rank test.

Results:

A total of 478 patients with previous surgical resection were eligible. Masaoka Stage was: I-86 (22%); II-87 (23%); III-107 (28%); and IV-106 (27%), respectively. Multivariate analysis demonstrated that gender (HR=1.4, p=0.03), stage (HR=1.3, p=3×), TC (HR=1.6, p=0.03) and PORT (HR=1.6, p=0.002) were significantly associated with PFS. Age (HR=1.1, p=4×), TC (HR=3.2, p=3×), stage (HR=1.4, p=0.003) were associated with OS.

PORT was given to 126 (26%) patients. Propensity score matching based on independent prognostic factors identified 99 patients for PORT, matched to 285 patients without. The 5-/10-year intra-thoracic progression free rates were 77%/69% and 85%/68%, for patients with and without PORT (p=0.009), respectively. The 5-/10-year PFS rates were 39%/18% and 61%/32%, for patients with and without PORT (p=0.002), respectively. The median survival, 5-/10-year OS rates for patients treated with PORT were 150 (95%CI 111~277) months, 87%/57% and respectively, compared to 192 months (95%CI 167~279), 88%/69% for patients receiving surgery alone (p = 0.13).

Conclusions:

This matched-paired analysis from a single institution suggests that PORT does not impact the PFS or OS in a selected population of resected TET.

Translational/Clinical Research Faculty

POSTER #79

STEREOTACTIC BODY RADIATION THERAPY MAY GENERATE COMPARABLE SURVIVAL TO SURGERY IN TREATING HEPATOCELLULAR CARCINOMA (HCC): RESULTS OF 756 PATIENTS

Feng-Ming (Spring) Kong[1], Yong Zang[2], Wenhu Pi[1], David Long[1], Susannah Ellsworth[1], Naoyuki Saito[1], Marwan Ghabril[3], Marco A Lacerda[3], David W Agarwal[4], Bert O'Neil[5], Mark Tann[4], Richard Zellars[1], Mary Maluccio[6]

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Background: Stereotactic Body Radiation Therapy (SBRT) has emerged as a viable treatment option in patients with hepatocellular carcinoma (HCC). This study aimed to compare survival outcomes after SBRT with other front line local treatments for HCC.

Methods: This is a retrospective analysis of patients identified through our cancer registry from 2000 to 2016. Patients treated with any local therapy alone were eligible: 1) SBRT, 2) surgery, 3) conventional external beam radiation (CEBRT), and 4) other local therapies including brachytherapy. Patients treated with combined therapies such as SBRT plus liver transplant were excluded. The primary endpoint was overall survival which was estimated from the time of diagnosis. Differences between the groups were compared using log-rank test. The data are presented as median (95%CI).

Results: A total of 756 patients with a median follow-up of 45 months (mo) met the selection criteria: 116, 380, 43, and 217 patients received SBRT, surgery, CEBRT, and other local treatment, respectively. Median age was 61, 60, 61 and 60 years, respectively. The median overall survival/3 year overall survival rate were 49 (32-66) mo /53% (44-65%) for patients treated with SBRT, which were not significantly different from 75 (57-94) mo /63% (58-69%) of surgery (p=0.27), non-significantly better than 22 (13-31) mo /41% (27-60%) of CEBRT (p=0.13), significantly better than 15 (13-20) mo /26% (20-34%) of other local treatments (p=3×¿10¿^(-7)). After adjusting for significant prognostic factors including age, race, status of tobacco abuse, history of alcohol use, tumor size, histology grade and stage, the survival outcomes of SBRT remained to be insignificantly different from surgery (HR=0.8, p=0.2), have a trend of significant difference from CEBRT (HR=1.4, p=0.1) and remarkably superior to that of other local treatments (HR=1.8, p=2×¿10¿^(-4)).

Conclusions: This study suggests that SBRT is an excellent front line option for HCC, potentially comparable to surgical resection and associated with longer survival than other front line local treatments. Randomized studies are needed to validate these findings.

Translational/Clinical Research ***Faculty***

POSTER #80

RADIATION TO THE NORMAL LUNG MAY BE AN IMPORTANT RISK FACTOR FOR SURVIVAL AFTER STEREOTACTIC BODY RADIATION THERAPY IN NON-SMALL CELL LUNG CANCER

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Background: Stereotactic body radiation therapy (SBRT) provides an outstanding local tumor control is a treatment of choice in patients with early stage medically inoperable non-small cell lung cancer (NSCLC). However, long-term survival after SBRT remained suboptimal which cannot be fully explained by comorbidity, distant cancer progression or commonly known toxicities. We hypothesize that there is an unidentified cause of death, which may be related to high radiation dose to normal tissue, especially to the lung. To test this hypothesis, we examined clinical and lung dosimetric factors associated with overall survival in early stage NSCLC treated with SBRT.

Methods: A total of 536 consecutive NSCLC patients received lung SBRT between 2002 and 2015 formed the base of this study population. Patients received one course of SBRT for primary treatment and with retrievable dosimetric data in our modern treatment planning system were eligible. The primary endpoint was overall survival, calculated from the start of SBRT. Clinical factors included age, gender, race, tobacco history, respiratory and cardiovascular comorbidity, tumor lobar location, histology, T stage, gross tumor volume (GTV), planning target volume (PTV), and prescription dose. Dosimetric factors of the lung were computed with exclusion of gross tumor volume (GTV) of either ipsilateral lung or both lungs, which were contoured according to the RTOG atlas.

Results: A total of 276 patients with NSCLC met criteria. The median follow-up were 47 months (95% CI 41-53 months). The median survival time was 33 months (95% CI: 25-42 months). The 2-year, 3-year and 5-year survival rates were 63%, 53% and 45%, respectively. Univariate analysis demonstrated that age (HR=1.02, p=0.04), gender (HR=0.75 for female, p=0.07), tumor T stage (HR=1.3 for T2, 2.5 for T3, using T1 as the reference, p=0.10), GTV (HR=1.01, p<0.001), PTV (HR=1.01, p<0.001), mean dose (HR=1.2, p<0.001), V5 (HR=1.02, p=0.03), V10 (HR=1.03, p=0.004), V20 (HR=1.1, p<0.001) of total lung were significant or borderline significant factors. Tobacco history, respiratory and cardiovascular comorbidity, tumor lobar location, histology, and physical prescription dose were not significant (all p>0.1). Dosimetric parameters of ipsilateral lung, presence of pneumonitis and fibrosis were not associated with survival (P>0.1). Under multivariate analysis, age (p=0.03), GTV (p=0.02), mean lung dose (p=0.01), V5 (p=0.05), and V20 (p=0.003) of total lung were significantly correlated with survival.

Conclusions: This study demonstrated at the first time that lung dosimetric factors are independent factors correlated with overall survival. This data suggests importance of further normal tissue sparing during SBRT planning. Further studies are required to understand the mechanism of this phenomenon.

Background: Stereotactic body radiation therapy (SBRT) provides an outstanding local tumor control is a treatment of choice in patients with early stage medically inoperable non-small cell lung cancer (NSCLC). However, long-term survival after SBRT remained suboptimal which cannot be fully explained by comorbidity, distant cancer progression or commonly known toxicities. We hypothesize that there is an unidentified cause of death, which may be related to high radiation dose to normal tissue, especially to the lung. To test this hypothesis, we examined clinical and lung dosimetric factors associated with overall survival in early stage NSCLC treated with SBRT.

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Conclusions: This study demonstrated at the first time that lung dosimetric factors are independent factors correlated with overall survival. This data suggests importance of further normal tissue sparing during SBRT planning. Further studies are required to understand the mechanism of this phenomenon.

Translational/Clinical Research Faculty

POSTER #81

RISK FACTORS FOR RADIATION-INDUCED LUNG TOXICITY AFTER STEREOTACTIC BODY RADIATION THERAPY IN PATIENTS WITH NON-SMALL CELL LUNG CANCER

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Purpose/Objective(s): Radiation-induced lung toxicity (RILT) is a potential fatal toxicity for stereotactic body radiation therapy (SBRT) for medically inoperable non-small cell lung cancer (NSCLC). This study aimed to examine the risk of RILT in a large institutional series of NSCLC patients and identify the dosimetric risk factors that predict RILT in order to help safe SBRT planning.

Materials/Methods: Patients with early-stage or recurrent NSCLC who received lung SBRT between July 2002 and June 2015 formed the study population. The primary endpoint was RILT, including pneumonitis (RP) and fibrosis (RF), which was diagnosed and graded retrospectively according to RTOG1106. Onset time of RILT, either RP or RF, was calculated from the start of SBRT. Lungs were contoured consistently by one radiation oncologist according to RTOG atlas for organs at risk. Dosimetric factors were computed with exclusion of gross tumor volume (GTV) of either ipsilateral lung or total lungs. Risk factors of interest included age, gender, smoking status, pathological type, tumor stage, treatment history, O2 use, baseline dyspnea, tumor location, prescription dose, GTV, planning target volume (PTV) and lung dosimetric parameters.

Results: A total of 366 patients with retrievable SBRT plans were eligible. The median follow-up was 55 months (95% CI: 47-64 months). There were 94 (27.4%), 123 (36.2%) and 169 (49.7%) events of RP, RF and RILT, respectively. There were 111(32.6%), 24(9.7%), 24(6.6%) and 1(0.3%) for G1, G2, G3 and G4 RILT, respectively. Of the patient and tumor factors tested, pulmonary co-morbidity ($p=0.007$), tumor located in left lung ($p=0.043$), previous radiation of thorax ($p=0.002$), O2 use ($p=0.048$) and baseline dyspnea ($p=0.011$) were significant for G2+RP, but only previous radiation of thorax was significant in multivariable analysis. Previous radiation of thorax ($p<0.001$) and baseline dyspnea ($p=0.015$) were significant for G2+RF in both univariable and multivariable analysis. Pulmonary co-morbidity ($p=0.004$), previous radiation of thorax ($p<0.001$) and baseline dyspnea ($p<0.001$) were significant risk factors for G2+RILT in both univariable and multivariable analysis. Of the dosimetric parameters factors analysis, V10 ($p=0.039$), V20 ($p=0.019$) of total lungs, mean lung dose (MLD) ($p=0.041$), V5 ($p=0.024$), V10 ($p=0.028$) and V20 ($p=0.027$) of ipsilateral lung were significantly associated with G1+ RP, but only V10, V20 of total lungs were significant in multivariable analysis. V5 ($p=0.023$), V10 ($p=0.037$) of ipsilateral lung were significant for G2+RP, but had no significance in multivariable analysis. MLD ($p=0.025$), V5 ($p=0.038$), V10 ($p=0.032$) of ipsilateral lung were significantly associated with G1+ RF, but had no significance in multivariable analysis. There was only a trend toward significance for V10 of total lungs ($p=0.054$) predictive of G2+RF. V5 ($p=0.025$), V10 ($p=0.015$), V20 ($p=0.026$) of total lungs, mean lung dose (MLD) ($p=0.007$), V5 ($p=0.006$), V10 ($p=0.007$) and V20 ($p=0.020$) of ipsilateral lung were significant for G1+RILT, but only V20 of total lungs was significant in multivariable analysis. No dosimetric factors were significant for G2+ RILT.

Conclusion: Previous radiation of thorax and baseline dyspnea were significant adverse risk factors for symptomatic RILT. Dosimetrically, V10, V20 of total lungs were predictive for RP, but only V20 of total lungs is a significant for RILT. This study represents one of the largest series reporting RILT after SBRT with comprehensive analysis of dosimetric risk factors for both ipsilateral lung and total lungs, with the lung organ consistently contoured per RTOG atlas.

Translational/Clinical Research Faculty

POSTER #82

SURVIVAL IN PATIENTS WITH HEPATOCELLULAR CARCINOMA (HCC): A REPORT OF 1444 PATIENTS

Mary Maluccio[1], Yong Zang[2], Wenhu Pi[3], Mark Tann[4], Chandrashekhar Kubal[1], Marco Lacerda[5], Bert O'Neil[6], David Agarwal[4], Feng-Ming (Spring) Kong[3]

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Background: The evolution of treatment for HCC has seen novel therapies emerge as front line treatment alternatives. The aim of this study was to report survival in HCC patients treated within the context of a robust multidisciplinary program and to identify patient and tumor specific factors that direct patient centered treatment decisions and optimize outcome.

Methods: This is retrospective analysis of medical records identified through the cancer registry at our institution from 2000 to 2016. Variables analyzed for survival significance included patient factors (age, gender, race, tobacco history, alcohol history, and marital status), tumor factors (tumor size, histology grade, AFP, SEER stage, clinical and pathologic stage). Survival was estimated from the time of diagnosis to the last contact.

Results: A total of 1444 consecutive patients with confirmed HCC were eligible for this analysis. Median follow-up was 45 months. Median survival was 18 months (95% CI: 11-25 months). The overall 1-, 3-, and 5 year survival rates were 63, 40, and 35%, respectively. Significant prognostic parameters were tobacco history (HR=1.2, p=0.03), SEER stage (HR=2.3, p=2×¿10¿^(-16), local as the reference), clinical stage (HR=1.1, p=4×¿10¿^(-5)) and pathologic stage (HR=1.2, p=3×¿10¿^(-9)). Of a total of 380 patients resected, median and 3 year survival were 75 months (95% CI: 57-94) and 63% (95% CI: 58-69%). The only significant prognostic parameter associated with survival with resection patients was SEER stage (HR=1.7, p=0.002). The 5 year survival for all vs resection were 44% (95% CI: 40-48) /59% (95% CI: 53-65), 21% (95% CI: 17-27) /36% (95% CI: 24-54), and 11% (95% CI: 5-20) /25% (95% CI: 6-100) for localized, regional, and distant disease, respectively.

Conclusions: Survival has improved for patients with HCC due to an increased number of available options and better methods to identify tumor and patients specific variables that individualize care. The significance of SEER stage suggests that early detection remains critical for survival.

Translational/Clinical Research ***Faculty***

POSTER #83

REFINING BREAST CANCER CHARACTERIZATION THROUGH SINGLE CELL ANALYSIS OF EX VIVO REPROGRAMMED TUMOR AND ADJACENT NORMAL CELLS

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There is a need to individualize assays for tumor molecular phenotyping, given variations in the differentiation status of tumor and normal tissues in different patients. To address this, we performed single-cell genomics of breast tumors and adjacent normal cells propagated for a short duration under growth conditions that enable epithelial reprogramming. Cells analyzed were either unselected for a specific subpopulation or phenotypically defined as undifferentiated and highly clonogenic ALDH+/CD49f+/EpCAM+ luminal progenitors, which express both basal cell and luminal cell-enriched genes. We analyzed 420 tumor cells and 284 adjacent normal cells for expression of 93 genes that included a PAM50 intrinsic subtype classifier and stemness-related genes. ALDH+/CD49f+/EpCAM+ tumor and normal cells clustered differently compared to unselected tumor and normal cells. PAM50 gene-set analyses of ALDH+/CD49f+/EpCAM+ populations efficiently identified major and minor clones of tumor cells, with the major clone resembling clinical parameters of the tumor. Similarly, a stemness-associated gene set identified clones with divergent stemness pathway activation within the same tumor. This refined expression profiling technique distinguished genes truly deregulated in cancer from genes that identify cellular precursors of tumors. Collectively, the assays presented here enable more precise identification of cancer-deregulated genes, allow for early identification of therapeutically targetable tumor cell subpopulations, and ultimately provide a refinement of precision therapeutics for cancer treatment.

Translational/Clinical Research ***Faculty***

POSTER #84

PREDICTING RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN BREAST CANCER USING DIFFUSE OPTICAL SPECTROSCOPY

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Over 20% of the nearly 200,000 women diagnosed with invasive breast cancer in the US each year will receive pre-surgical neoadjuvant chemotherapy (NAC). This fraction has been steadily rising for 10 years. NAC treatment allows oncologists to monitor individual tumor response during treatment, and thus modify treatment to optimize efficacy. Established and emerging medical imaging modalities such as ultrasound, PET, MRI, and diffuse optical spectroscopy (DOS) are all being actively investigated for their ability to assess response to NAC noninvasively and with greater accuracy than palpation (the current standard-of-care). Of these modalities, DOS is advantageous because it is a safe, low-cost functional technique that can be performed frequently at the point of care and does not require contrast injection or exposure to ionizing radiation. DOS, based upon the absorption of near-infrared light in tissue, provides a quantitative measure of tissue functional components oxy- and deoxyhemoglobin, water, and lipid, which are directly related to breast tissue metabolism and vascular characteristics.

In this presentation, we discuss how our group is contributing to the development and validation of DOS technology, including our role in completing the first multi-center clinical study of DOS technology sponsored by an independent oncology research group (ECOG-ACRIN 6691). This prospective trial was designed to evaluate whether changes in a DOS-derived imaging endpoint, the tissue optical index (TOI), predict pathologic complete response (pCR) in women undergoing NAC for breast cancer. DOS instruments were constructed and delivered to six institutions. 60 subjects with >2 cm breast tumors were enrolled and DOS images were acquired on both breasts at up to four times before or during NAC treatment: baseline, 1-week, mid-point, and completion. Of the 34 subjects with complete, evaluable data, 10 (29%) achieved pCR as determined by central pathology review. The percent change in tumor-to-normal TOI ratio ($\%TOI_{TN}$) from baseline to mid-therapy ranged from -82% to 321%, with a median of -36%. A negative change in $\%TOI_{TN}$ indicates a favorable treatment response. Using pCR as the reference standard and receiver operating characteristic curve (ROC) methodology, the $\%TOI_{TN}$ area under the ROC curve (AUC) was 0.60 (95% CI, 0.39-0.81). In the cohort of 17 patients with baseline tumor oxygen saturation greater than the 77% population median, $\%TOI_{TN}$ AUC improved to 0.83 (95% CI, 0.63-1.00). This study demonstrated the promising utility of DOS for predicting NAC response.

We conclude with a brief summary of other applications for DOS under investigation in breast oncology including differential diagnosis of suspicious lesions, screening in dense breast tissue, breast density quantification, and measuring response to hormonal treatment agents such as tamoxifen.

Translational/Clinical Research ***Faculty***

POSTER #85

FACTORS ASSOCIATED WITH OVERALL SURVIVAL AFTER RADIATION THERAPY IN PATIENTS WITH HEPATOCELLULAR CARCINOMA (HCC)

Wenhu Pi[1], Young Zang[1], Hong Zhang[1], Huan Yao[1], Susannah Ellsworth[2], David Long[1], Weili Wang[1], Jian-Yue Jin[1], Richard Zellars[1], Mary Alice Maluccio[3], Feng-Ming (Spring) Kong[1]

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Background

The role of radiation therapy (RT) is evolving in patients with unresectable and inoperablehepatocellular carcinoma (HCC). This study is to 1) evaluate the efficacy of RT for HCC patients, and 2) identify factors predictive of survival in patients treated with RT.

Methods

HCC patients from 2000 to 2016 in our institution were collected from the cancer registry system and studied retrospectively. Patients with any form of external beam radiation including stereotactic body radiotherapy (SBRT) and 3D conformal Radiation therapy (3DCRT) were eligible. The commonly used SBRT regimens were 48Gy in 4 fractions, 50 Gy in 5 fractions and 60 Gy in 3 fractions. The median dose of 3DCRT was 38 Gy (range 20-60 Gy). Age, gender, race, tobacco history, alcohol history, primary insurance payer, marital status, tumor size, histology grade, AFP level, SEER stage, clinical stage and pathologic stage, total dose, integral dose of the total dose, gross tumor volume (GTV), mean dose of GTV, planning target volume (PTV) and mean dose of PTV, liver volume, number of fraction, tumor biologically effective dose (BED), mean liver dose, SBRT and 3DCRT were tested for their association with survival. The primary endpoint is overall survival which was calculated from the first time of treatment to the last time of contact.

Results

A total of 184 patients received external beam RT were included in this analysis: 127 with SBRT and 57 with 3DCRT. The median age was 61 years and the median follow-up was 37 months (95% CI 31-60), respectively. The 2- and 5- year survival rates were 61% (95% CI 54-69%) and 40% (95% CI 32-50%) respectively. Under the univariate analysis, tobacco history (current cigarette smoker, HR=3.9, p=0.03), SEER summary stage (distant, HR=8.3, p=3×), tumor size per 1 cm (HR=1.1, p=0.0006), total dose (HR=0.96, p=0.0006), integral dose (HR=1.3, p=1×), PTV (HR=1.3, p=9×), GTV(HR=1.001, $p=4.27x10^{-6}$), mean liver dose (HR=1.1, p=0.01), number of fraction (HR=1.1, p=0.002), BED (HR=1.008, p=3x) and 3DCRT (HR = 2.3, $p = 9.6x10^{-5}$) were significant factors for overall survival. Under multivariate analysis, PTV (HR=1.6, p=0.021) remained significant. Thenumber of fraction had a borderline significance (HR=1.1, p=0.06), mean liver dose and other variables were not (all p>0.1).

Of all 184 patients, 15 patients received liver transplant after RT (10 SBRT, 5 3DCRT). The median OS was 33 months (95% CI 27-59) for patients without transplant and 35 months for patients with transplant (HR=0.4, p=0.05, patients without transplant as reference).

Conclusions

Radiation therapy provides reasonable long term outcomes with 40% 5-year survival in this populations. Negative impact of PTV on survival suggests importance of diagnosing and treating smaller tumor and

potential benefit of high technology to shrink the PTV margin.

Translational/Clinical Research Faculty

POSTER #86

PROGNOSTIC BIOMARKERS FOR THE DEVELOPMENT OF RESPIRATORY FAILURE POST ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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Background/Objectives: Currently, there is not a method for predicting respiratory failure in the allogeneic hematopoietic cell transplant (HCT) recipient. Biomarkers may be effective for risk recognition, understanding etiology, and serving as therapeutic targets. The study's aim was to identify prognostic biomarkers for the development of respiratory failure.

Design/Methods: This is a single center analysis of an existing cohort from a clinico-biologic repository.Plasma samples at days 7, 14, 21, and 30 post-HCT were used to measure four proteins by ELISA: STimulation-2 (ST2), the IL33 receptor, interleukin 6 (IL6), tumor necrosis factor receptor 1 (TNFR1) and osteopontin (OPN).Data is presented in medians and were compared using Wilcoxon rank sum test. ROC curves were also done for each protein.

Results:A total of 122 adult and pediatric allogeneic HCT recipients were included with 24.6% (n=30) developing respiratory failure within 1 year post-transplant.The median day to respiratory failure was 27.0. The medians of all four proteins were significantly higher in the group that developed respiratory failure at multiple time points:
Day 7 Post HCT: ST2 [57.0 vs 24.9, p-0.003], IL6 [100.0 vs 39.2] p=0.002, TNFR1 [4184.0 vs 2425.6 p=0.02]

Day 14 Post HCT: ST2 [58.5 vs 22.0, p-0.003], IL6 [91.8 vs 32.5 p=0.001], TNFR15499.2 vs 3121.9 p=0.006], OPN [340.0 vs 277.8, p=0.04]

Day 21 Post HCT: ST2 [50.0 vs 25.0, p<0.0001], IL6 [62.8, 21.0, p=0.006], TNFR1 [5793.0 vs 3314.0 p=0.003], OPN [332.0 vs 220.0, p=0.003]

Day 30 Post HCT: ST2 [40.0 vs 20.0, p<0.0001], IL6 [53.0, 18.1, p=0.006], TNFR1 [5518.0 vs 3024.6 p=0.001], OPN [275.0 vs 185.0, p=0.02]

ROC curves indicate that these proteins allowed for good discrimination for the development of respiratory failure within 45 days of transplant (AUCs: ST2=0.83, TNFR1=0.78, OPN=0.78, and IL6=0.73).

Conclusions:ST2, IL6, TNFR1, and OPN were all significantly elevated at multiple time points in those that develop respiratory failure post-allogeneic HCT.

Translational/Clinical Research ***Faculty***

POSTER #87

EFFECTS OF THE ANTI-ESTROGEN ENDOXIFEN ON THE MUSCULOSKELETAL SYSTEM AND IMPLICATIONS FOR THE TUMOR MICROENVIRONMENT

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Adjuvant endocrine therapy is a standard treatment for postmenopausal women with ER-positive breast cancer. Unfortunately, between 25-50% of women treated with endocrine therapies develop musculoskeletal toxicities that result in treatment discontinuation. Previous studies in our laboratory demonstrated that aromatase inhibitor (AI) treatment caused bone loss and skeletal muscle weakness in mice, recapitulating effects observed in cancer patients. We also demonstrated that prevention of AI-induced osteoclastic bone resorption using a bisphosphonate attenuated ER-negative breast cancer bone metastases and improved muscle function. These preclinical findings highlight the bone microenvironment as a modulator of tumor growth locally and muscle function systemically. Because muscle weakness is also commonly reported in women treated with selective estrogen receptor modulators (SERMs), we compared musculoskeletal effects of AI with a bone-sparing SERM endoxifen in a non-tumor model. Endoxifen (Endx), an active metabolite of tamoxifen, is currently in phase I trials for ER+ advanced breast cancer and little is known of its effects on the musculoskeletal system. Mature female C57BL/6 mice underwent sham surgery or ovariectomy (OVX) and were treated daily with vehicle, the AI letrozole (Let), or Endx. After eight weeks, changes in cancellous and cortical bone indices were assessed by μCT and muscle contractility of the extensor digitalis longus (EDL) was measured *ex vivo*. Bone volume fraction (BV/TV) decreased by 50% in OVX-vehicle and OVX-AI mice ($p<0.05$), whereas BV/TV increased threefold in Endx mice relative to sham-vehicle ($p<0.0001$). Periosteal and endosteal expansion of cortical bone was inhibited by Endx evidenced by a decrease in medullary area, total cortical cross sectional area, and polar moment of inertia relative to sham- and OVX-vehicle ($p<0.0001$). At the termination of the study, muscle-specific force was lower in OVX-Endx mice relative to OVX-vehicle and OVX-AI mice ($p<0.05$), indicating that SERM-induced muscle weakness may be independent of bone resorption. Histological and biochemical assessment of skeletal muscle will be performed to determine a mechanism for muscle weakness in Endx-treated mice. Ongoing studies will determine how Endx-driven changes in cancellous and cortical bone morphology impact the mechanical strength of bone, and how these changes to the bone microenvironment impact breast cancer metastasis to the skeleton.

Translational/Clinical Research ***Faculty***

POSTER #88

USING ROC ANALYSIS TO DETERMINE THE OPTIMAL LUNG DOSIMETRIC PARAMETER AS THE RISK FACTOR OF SURVIVAL FOR SBRT IN NSCLC PATIENTS

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Purpose: Stereotactic body radiation therapy (SBRT), the standard of care for inoperative early stage non-small cell lung cancer (NSCLC), provides an outstanding tumor control with minimum toxicity. However, the overall survival (OS) remains suboptimal. We have demonstrated that mean lung dose is a significant risk factor for OS. To understand the underlying mechanism of why lung dose affect OS, this study aimed to determine the optimal lung dosimetric parameter that best correlates with OS.

Methods: A total of 245 early-stage NSCLC patients with SBRT between July 2002 and June 2015 were included in the study. Lung dosimetric parameters were computed as the generalized equivalent uniform dose (gEUD) of various lung definitions including ipsilateral lung (IL), contralateral lung (CL) or total lungs (TL) excluding gross tumor volume (GTV) and planning target volume (PTV). In addition, gEUD with "a" = 0.1 to 6.0 were also computed. Area under curve (AUC) was used to assess model accuracy of dosimetric parameters to select the optimal lung dosimetric parameter.

Results: The gEUDs of variously defined lungs were significant for OS ($P<0.05$). The AUCs for gEUD of IL-GTV, IL-PTV, CL-GTV, TL-GTV and TL-PTV were 0.64, 0.61, 0.59, 0.61 and 0.61, respectively. The AUCs for gEUD of IL-GTV with "a"= 0.1, 0.5, 1.0 1.5, 2.0, 2.5, 2.8, 3.0, 3.5, 4.0 and 6.0 were 0.56, 0.64, 0.65, 0.64, 0.63, 0.64, 0.67, 0.66, 0.66, 0.64 and 0.61 respectively. "a" =2.8-3.5 appeared to have the highest AUC, i.e. the best predictive value. The superiority of IL-GTV over others were significant throughout the course of follow-up.

Conclusion: The gEUD with a =2.8-3.5 for ipsilateral lung excluding GTV appears to be the best lung parameter to predict the survival of patients treated with SBRT. This result suggests that the lung dosimetry around the GTV affects the survival in NSCLC SBRT. Future SBRT plan should use technology to minimize gEUD of IL-GTV to improve survival.

Translational/Clinical Research ***Faculty***

POSTER #89

FACTORS ASSOCIATED WITH SURVIVAL IN PATIENTS WITH NON-SMALL CELL LUNG CANCER FROM A SINGLE INSTITUTION STUDY OF 3569 PATIENTS

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Background: Non-small cell lung cancer (NSCLC) constitutes more than one quarter of cancer deaths in the United States. The primary aim of this study is to identify risk factors and build a model for overall survival prediction based on patient and tumor factors from a large single institution database.

Methods: The study population was taken from our cancer registry of 8620 lung cancer patients treated in the Indiana University Health System between 2000 and 2016. Patient and tumor factors were tested for their correlation with survival. The patient records with loss to follow up were censored. The survival analysis was performed using a multivariable cox proportional hazards regression model with R. The accuracy of the models were assessed by 8 folder cross validation.

Results: After excluding other histologies and duplicate registered cases**,** 3569 patients with confirmed NSCLC and complete staging data were eligible for this analysis. Median follow-up was 16.7 months in all patients and 41.8 months for alive patients. The median/5-year OS were 34.3 months/51.3%, 27.1 months/44.0% (HR = 1.2, p = 0.03), 14.2 months/18.0% (HR = 2.4, p = 2e-16) and 6.3 months/6.7% (HR = 4.9, p = 2e-16) for stage I, II, III and IV, respectively. Age, gender, race, marital status, smoking status, tumor size, stage, year of diagnosis and treating hospital were significant factors for survival (all $p<0.001$). Using these significant factors, a multivariate cox proportional hazard regression model was built, with the stage adjusted by gender (HR = 0.8 for female, p = 9.52e-7), smoking status (HR = 1.2 for current smoker, p = 0.001), age (HR = 1.03, p = 2e-16), marital status (HR = 1.2 for single, p = 0.001), tumor size (HR = 1.008, p = 2e-16), year of diagnosis (HR = 0.97 for more recent year, p = 1.3e-9) and hospital (HR = 0.8 for university hospital, p = 0.0001). This model was further tested with 8 folders cross validation, to have 66% accuracy which was slightly improved from 63% of using staging group alone.

Conclusions: Many patient factors contribute to survival in patients with NSCLC. A model of combining independent patient and tumor factors provides only modest accuracy. Further study to integrate treatment modality and biologic factors is warranted to generate a more accurate survival model for precision medicine in NSCLC.

Translational/Clinical Research ***Faculty***

POSTER #90

CACHEXIA AVATARS MODELING SYSTEMIC DISEASE IN PANCREATIC CANCER SHOW IL-6 AND MUSCLE WASTING ASSOCIATE WITH MORTALITY

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Background: Cancer cachexia, or unintentional weight loss, is a metabolic and inflammatory derangement that reduces quality and length of life and diminishes response to therapy. Treatment options are limited, due to the complexity of the disease and the lack of success of clinical trials. There is a general lack of knowledge of whether there are heterogeneous mechanisms and phenotypes in cachexia, and whether cachexia is driven by the same factors within or across tumor types. Among patients with pancreatic ductal adenocarcinoma (PDAC), over 80% suffer cachexia. Given the high penetrance of cachexia in this patient population, we sought to characterize the systemic disease caused by individual tumors to begin to define the phenotypic and molecular spectrum in this disease state.

Methods: In an IRB-approved study, we collected tumor from patients undergoing surgery for PDAC and implanted fragments into the pancreata of NSG mice (P0). Fragments that grew into tumors were passaged into a second host (P1). P1 tumor fragments were implanted in the pancreases of 7 recipient mice (P2) for evaluation of cachexia potential. At least 3 sex and age-matched mice were kept in the same cages as controls. Ten tumor lines were evaluated in this fashion. Histopathology from 8 of these lines was consistent with PDAC. Two lines induced lymphoma and were excluded. Mice were kept until they displayed a body condition score of 8 or 9 on a scale of 0-9, with 0=normal and 9=moribund. Mice were euthanized and body composition and organ size were determined.

Results: The eight independent PDAC patient-derived orthotopic tumor lines elicited a spectrum of systemic disease and mortality, with the most virulent line causing death at 26 days and the least at 176 days ($P<0.001$). Effects on organ, muscle, fat and carcass mass varied, as did effects on cardiac function. Neither tumor size nor changes in fat, heart, liver, spleen, or total body weight correlated with mortality. However, loss of quadriceps mass correlated with tiem to euthanasia ($r^2=0.822$, $P=0.0019$). Because Interleukin-6 (IL-6) is a known mediator of muscle wasting in cachexia, we evaluated tumors and plasma for IL-6. All the patient tumors and xenografts stained positive for IL-6, either in the tumor or stromal compartments or both. However, only the mice with most virulent tumor lines showed elevations of plasma IL-6. Indeed, human IL-6 levels correlated with mortality ($r^2= -0.873$, $P=0.06$), while there was a trend for murine IL-6 levels ($r^2= -0.714$, $P=.088$).

Conclusions: These results indicate that tumor-intrinsic qualities in PDAC can produce diverse cachexia phenotypes, suggesting heterogeneity of cachexia drivers and mechanisms within a single tumor type. Identifying the factors mediating these differences might be essential to provide precise, effective therapy. Furthermore, studies using our cachexia “avatar” hospital could test efficacy of anti-cachexia therapies.

Translational/Clinical Research ***Faculty***

POSTER #91

THE ROLE OF THE INNATE IMMUNE RESPONSE IN ERG POSITIVE PROSTATE CANCER

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Prostate cancer is responsible for the second most cancer-related deaths in American men. A common characteristic occurring in approximately 50% of prostate cancers is the presence of a gene fusion between the highly active, androgen driven promoter region of the TMPRSS2 gene and the normally unexpressed transcription factor, ERG. This fusion results in overexpression of ERG, resulting in increased migration and invasion, which are crucial for the development of more aggressive and metastatic cancer. Screening an shRNA library using the trans-well migration assay revealed knockdowns of the innate immune response produce less migratory cells, and treatment of prostate cancer cells with an inhibitor of the innate immune response results in decreased protein expression of the oncogenic ERG transcription factor. The objective of this work is to explore the mechanism by which this drug decreases ERG expression and to determine any drug-mediated changes in oncogenic phenotypes.

Western blot and qRT-PCR data indicate a reduction of both ERG protein and mRNA transcripts in the VCaP prostate cancer cell line upon treatment, suggesting the expression is altered at the transcriptional level. Combination treatment with the innate immune response inhibitor and the proteasome inhibitor MG132 failed to produce a rescue in ERG protein expression, providing evidence that drug treatment does not affect protein stability. Trans-well migration assays suggest this drug can reduce migration in ERG expressing prostate cells, but has no effect on ERG negative prostate cancer cell lines. This trend is also observed in scratch migration assays. Clonogenic survival assays have revealed that this inhibitor can also specifically reduce the formation of colonies of ERG expressing cells. Finally, growth curves indicate that there is no effect of drug treatment on proliferation.

These data suggest that inhibition of the innate immune response can downregulate transcription of the oncogenic transcription factor, ERG, in prostate cell lines and reduce their tumorigenicity *in vitro*. Because this drug targets ERG positive cells, it may be a useful and clinically relevant treatment for many prostate cancer patients. Further experiments investigating the drug's cytotoxicity as well as its effectiveness on ERG positive patient-derived xenografts are underway.

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

POSTER #92

CONSTRUCTING BAYESIAN GENE CONTROL NETWORK FOR PRIORITIZATION OF DRUG TARGETS IN PANCREATIC DUCTAL ADENOCARCINOMA

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Background

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive cancer with dismal prognosis. PDAC responds poorly to most chemotherapeutic and target-drug agents. There is an urgently need, therefore, to identify distinguishable gene expression signatures for prioritization of drug targets.

Methods

In this study, we developed a Bayesian Network-based approach to predict PDAC-associated genes by transcriptome profiling. We constructed a cross-talking network to integrate tumors, human xenograft mouse models and cancer cells interactions in pancreatic cancer. We next extended the traditional Bayesian Network construction from a small scale to a whole genome by a random separation and connection algorithm, and used known pancreatic cancer genes as the seeds to find novel candidate target genes for PDAC across tumors, human xenograft mouse models and cancer cells.

Results

We validated our results by comparing high-degree nodes in constructed networks with genes in previously reported 12 core pathways of PDAC, in which two pathways are identified as enriched, the others have representative genes being altered. Moreover, we identified 1,375 dysregulated genes that are involved in new pathways, among which 244 play as hubs in constructed PDAC regulatory network. 9,960 genes are found not involved in any pathways, of which 747 genes act as hubs in constructed PDAC regulatory network. cancer associated known genes We subsequently cross-validated these hub genes among tumor-model, mouse xenograft model and cell-line PDAC model and eventually identified a set of 54 genes that shows strong concordance among the three. In addition, we demonstrated that top-ranked candidate genes were enriched for drug targets, and identified commonalities underlying top-ranked pancreatic genes through pathway analysis.

Conclusion

The update Bayesian Network-based approach is a useful tool or predicting disease correlated genes. The candidate pancreatic cancer-associated genes predicted by our data-driven approach have the potential to guide genetics-based anti-pancreatic drug discovery.

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

POSTER #93

NOVEL COMBINATION THERAPY OF DNA METHYLTRANSFERASE INHIBITOR GUADECITABINE AND PARP INHIBITOR TALAZOPARIB FOR BRCA-PROFICIENT HIGH-GRADE SEROUS OVARIAN CANCER

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Ovarian cancer recurrence has been shown to be associated with increased DNA damage response (DDR) mediated by poly-(ADP)-ribose polymerase 1/2 (PARP1/2), which can be therapeutically targeted by PARP inhibitors (PARPi). PARPi are indicated for platinum-responsive, BRCA-mutated high-grade serous ovarian cancer, but most ovarian cancer patients have BRCA-proficient disease. Our previous studies support a role for DNA methylation in chemoresistant ovarian cancer mediated by the enzyme DNA methyltransferase 1 (DNMT1), and report on a functional role for DNMT1 in DNA damage repair. We therefore hypothesized that combining a DNMTi and PARPi will impair BRCA-mediated DDR, resulting in cytotoxicity in ovarian cancer cells. A panel of ovarian cancer cell lines (A2780, platinum sensitive, BRCA1/2-wild type; A2780-cp and HeyC2, platinum resistant, BRCA1/2-wild type; high-grade serous Kuramochi, platinum resistant, BRCA2 mutant) was examined for cell growth using colony formation assays after treatment with DNMTi guadecitabine (low dose, 20-100nm) and PARPi talazoparib (1-10nm), alone or in combination. While talazoparib alone only marginally reduced colony formation in all cell lines (dose-dependent effect), combining guadecitabine with talazoparib further decreased ($P<0.05$) survival at all doses examined. To focus more specifically on BRCA status, we utilized two high-grade serous ovarian cancer cell lines ("PEO") derived from the same patient but harboring a mutant (PEO1) or wild type (PEO4) BRCA2 gene (Langdon et al, 1988; Sakai et al, 2009). Treatment with low-dose guadecitabine (20 nm, 3 days) increased ($P<0.05$) PARP levels (western blot analysis) as well as enzymatic activity ($P<0.05$; ELISA analysis), while talazoparib treatment alone increased ($P<0.05$) DNMT1 levels and decreased ($P<0.05$) PARP enzymatic activity. Treatment with guadecitabine or talazoparib alone had no effect on cell proliferation; however, combining the two drugs inhibited (>80%, $P<0.05$) PEO1 and PEO4 proliferation and increased (3-fold, $P<0.05$) apoptosis (caspase 3 cleavage) in both cell lines. Furthermore, co-administration guadecitabine (0.5mg/kg) and talazoparib (0.25mg/kg) to mice harboring BRCA2-wild type ovarian tumor xenografts decreased ($p<0.05$) tumor volume (>60%) and tumor weight (~70%) compared to control, respectively. In summary, combining a hypomethylating agent with a PARP inhibitor results in enhanced cytotoxicity in high-grade serous ovarian cancer cell lines harboring either wild type- or mutant-BRCA, indicating that the talazoparib-guadecitabine drug combination is effective regardless of BRCA-mediated DDR and may represent an effective treatment regimen for BRCA-related cancers.

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

POSTER #94

BRAIN METASTATIC MICROENVIRONMENT RESHAPES CANCER CELL METABOLISM THROUGH EPIGENETIC UP-REGULATION OF GLUTAMATE DECARBOXYLASE 1

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Cancer metabolism has been well documented to influence primary tumor development. Yet, the role of deregulated metabolism in metastasis progression is not well understood. Based on a distinct metabolic transcriptome profile in brain metastases compared to their primary tumor counterparts, we hypothesized that metabolic transcriptome shifting during metastatic evolution is crucial for metastatic success to the brain. Here we show that, despite a global down-regulation of multiple metabolic pathways in the brain metastatic tumors, the expression of the GABA metabolic pathway mediator glutamate decarboxylase 1 (GAD1) is significantly up-regulated. Using cell based co-culture models for different primary and brain metastatic microenvironments and *in vivo* brain metastasis models, we demonstrate that down-regulation of DNA methyltransferase 1 (DNMT1) induced by the brain microenvironment results in decreased GAD1 promoter methylation and subsequent up-regulation of GAD1 expression in tumor cells. To dynamically visualize the cellular metabolic responses mediated by GAD1, we utilized the Peredox biosensor to monitor the cytosolic NADH:NAD^+equilibrium in tumor cells using time-lapse imaging. By knocking down of GAD1 induced by primary glia cell co-culture, we abolished the capability of tumor cells to utilize extracellular glutamine, leading to an NADH accumulation in the cytosol and a more oxidative cellular status. Lastly, either loss of GAD1 genetically or targeting GABA metabolic pathway by the repurposing of a neurological drug, vigabatrin, results in a significant decrease in brain metastasis incidence. Taken together, our results demonstrated that brain microenvironment-specific metabolic shifting through GAD1 promoter de-methylation drives brain metastasis outgrowth.

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

POSTER #95

BI-EM: A FAST BI-CLUSTERING ALGORITHM TO DETECT CO-EXPRESSION GENES CROSSING MULTI-OMICS DATA SETS

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Background: High resolution microarrays and second-generation sequencing platforms are powerful tools to investigate genome-wide alterations in gene mutation, gene expression and proteomics associated with cancer. Combining these data, rather than exploiting them separately, can significantly increase the power of clinically relevant patients' sub-classifications. New approaches are urgently needed to determine common co-expression pattern across multi-omics datasets of patients.

Method:A novel statistical Bi-EM (multi-omics Bi-clustering based on Expected-Maximum (EM) algorithm) is proposed to detect co-expression genes based on mRNA and protein expression profiles. An EM process is used to identify clear bi-clustering signals compare to background values. A seed search strategy is used to detect correlated inner bi-cluster signal crossing mRNAs and proteins or tumors and cancer cells.

Result: The experiment simulation results show the proposed Bi-EM algorithm has much higher sensitivity and speed than eight prominent biclustering methods when applied two synthetic datasets; constant pattern and the scale-shifted pattern. In addition, Bi-EM algorithm is applied to breast cancer tumors to detect sub-classification specific patterns in both mRNA and protein. Based on gene/protein expression of Luminal A/B data, the largest bicluster includes 12 genes with similar expression across 219 samples. ER, ER (p118), and PGR have over expression of mRNA/protein in the bicluster. AR, BCL2, Cyclin E1 and IGFBP2 are also included in the bicluster which is concordance with clinical practice in Luminal A/B subtype.

Conclusion: In this study, probabilistic algorithm, Bi-EM, is constructed to search coherent patterns across multi-omics mRNA/protein simultaneously. Simulation results indicated our algorithm had high accuracy compare to other algorithms. Bi-EM is presented as an important sub-classifications pattern detection tool for multi-omic datasets of tumors.

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

POSTER #96

COMPARING THE FREQUENCY OF UPPER-EXTREMITY NEUROPATHY IN YOUNGER AND OLDER BREAST CANCER SURVIVORS DURING LONG-TERM SURVIVORSHIP

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Abstract

Background: Common treatment strategies for breast cancer such as surgery, chemotherapy, and radiation can put survivors at-risk for nerve damage (neuropathy) in their arms and hands following treatment. Current data indicates that a large percentage of women continue report symptoms of neuropathy such as numbness and tingling in their upper-extremities during the first three years post-treatment. However, the frequency of upper-extremity neuropathy in long-term survivorship (three or more years post-treatment) is poorly defined, making it difficult to coordinate care for this growing population. In addition, while recent studies have shown that symptoms like anxiety, fatigue, and depression can be more common for younger survivors (i.e., <45 years) than older survivors (<55 years) following treatment, studies exploring whether the frequency of upper-extremity neuropathy is different for younger and older survivors during long-term survivorship have yet to be performed.

Purpose: (1)To describe the frequency of upper-extremity neuropathy in breast cancer survivors three or more years post-treatment, (2) to compare the frequency of upper-extremity neuropathy between younger and older survivors during long-term survivorship.

Materials and Methods: Data for the retrospective analysis came from a recent cross-sectional study of quality of life in 505 younger and 622 older survivors (N=1,107) after treatment. Participants were three to nine-years post-treatment (mean: 6.0 ±1.5). All women were treated with breast surgery and Adriamycin, Cytoxan and Taxol (AC+T) chemotherapy regimen. Data on the frequency of four common neuropathy symptoms (burning, numbness, tingling and skin crawls) in the upper-extremities during the past week was collected from a questionnaire presented to women at their visit.

Results: Results of the analysis showed that an average of six years post-treatment, 57.8% of younger survivors and 49.2% of older survivors reported at least one upper-extremity neuropathy symptom in the during the past week. While younger survivors were more likely than older survivors to report neuropathy ($p<.000$), both groups reported nearly identical types of upper-extremity symptoms, primarily involving numbness or tingling.

Conclusions: Even years after treatment, upper-extremity neuropathy is common sequalae for survivors treated with surgery and chemotherapy. Clinicians working in the long-term survivorship setting should remain alert for upper-extremity neuropathy, especially in younger survivors, who may be more likely to present with symptoms than their older counterparts. Studies validating these findings in prospective cohorts of long-term survivors and evaluating the impact that these lingering upper-extremity symptoms have on breast cancer survivors are needed to deepen our understanding of how best to care for women during long-term survivorship.

Words: 405

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

POSTER #97

TUMOR-INDUCED STROMAL STAT1 ACCELERATES BREAST CANCER PROGRESSION BY DEREGULATING TISSUE HOMEOSTASIS

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Introduction
The tumor microenvironment (TME) – the dynamic tissue space in which the tumor exists – plays a significant role in tumor initiation and is a key contributor in cancer progression. Little is known about the tumor-induced changes in the adjacent tissue stroma. Herein, we sought to explore tumor-induced changes in the TME at the morphological and molecular level to further characterize cancer progression.

Methods
We injected PNA.Met1 cells into one of the inguinal mammary fat pads of female FVB mice with a vehicle control injection in the contralateral mammary fat pad. We assayed branching morphology by mammary whole mounting, morphology by immunohistochemistry, and changes in the epithelial hierarchy by mammosphere assay and fluorescent activated cell sorting (FACS). We performed comparative transcriptomics on the tumor-adjacent glands (TAGs) and the control glands.

Results
We show that TAGs display altered branch morphology, increased expression of aSMA particularly in myofibroblasts, and an increased capacity to form mammospheres in 2D suspension culture. FACS analysis showed that TAGs contain an increased number of Lin-CD24+/CD49+ enriched mammary gland stem cell (MaSCs) population, suggesting deregulated tissue homeostasis in TAGs. We conducted comparative transcriptomics on TAGs and contralateral control glands. Meta-analysis on differentially expressed genes from our RNA-seq dataset plus two breast cancer stromal patient microarray datasets identified shared upregulation of STAT1, which we verified in tumor-adjacent tissues. Knockdown of STAT1 in caveolin-deficient mouse embryonic fibroblasts (CAFs) cocultured with human breast cancer cells altered cancer cell proliferation, further suggesting the role of STAT1 as a stromal contributor of tumorigenesis. Furthermore, in our proof-of-concept in vivo experiment, co-treatment with fludarabine, a FDA-approved STAT1 activation inhibitor and DNA synthesis inhibitor, in combination with doxorubicin, showed enhanced therapeutic efficacy in treating mouse mammary gland tumors.

Conclusions
Our results demonstrate that stromal STAT1 expression could promote mammary tumor progression and is a potential therapeutic target for breast cancer.

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

POSTER #98

VENA CAVA-SPARING PIGGYBACK HEPATECTOMY IN LIVER TRANSPLANT PATIENTS WITH HEPATOCELLULAR CARCINOMA

Weston Bush

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Introduction:

Liver transplant (LT) patients with hepatocellular carcinoma (HCC) are at risk for post-transplant tumor recurrence. Risk of HCC recurrence is known to be associated with the size and number of tumors present within the liver. Close proximity of tumor to major vascular structures may also increase the risk of tumor recurrence. For that reason, most surgeons employ a conventional bicaval technique, replacing the entire vena cava as part of the LT. Our center has previously published data suggesting that the vena cava-sparing piggyback (PGB) technique can be safely used without affecting clinical outcomes. This study reviews a large number of LT patients with HCC to determine long-term outcomes of using the PGB technique, as well as the impact of tumor proximity to the vena cava on recurrence rates.

Methods:

The records of all adult patients undergoing liver transplant (LT) at a single center over a 15-year period were reviewed. Patients with HCC were extracted for further analysis. The operative records for all HCC patients were reviewed to determine if the CONV or PGB hepatectomy technique was utilized. Original computed tomography scans were reviewed to measure distance between the vena cava and the nearest tumor, and to determine which segments of the liver had tumor present. Outcomes included HCC recurrence and long-term patient survival. Cox regression 10-year patient survival was calculated.

Results:

There were 1722 LT patients, and 393 were found to have HCC (23%). Among these patients, 367 (93%) underwent LT with PGB technique, while 26 had CONV hepatectomy (7%). The PGB patients were older and had an older donor age, but had lower cold and warm ischemia time. The PGB patients were more likely to have HCC in segments adjacent to the vena cava (57% vs 34%, p=0.02), but the median distance to the nearest tumor was greater for the PGB group (45 vs 28mm, p=0.06). There was no significant difference in tumor recurrence between PGB and CONV (16% vs 19%, p=0.70), nor was there a difference by Cox regression in survival at 10-years (p=0.13). Predictors of recurrence included being outside Milan criteria, and increased tumor size and number, but not tumor distance to the vena cava.

Conclusion:

These results demonstrate no significant difference in clinical outcomes between the PGB and CONV surgical techniques in LT patients with HCC. Tumor presence near the vena cava was not associated with increased risk of HCC recurrence.

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

POSTER #99

POST LIVER TRANSPLANT CANCER RISK IN PATIENTS RECEIVING ANTIBODY-BASED IMMUNOSUPPRESSION INDUCTION

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Introduction:

Solid organ transplant patients have an increased risk of cancer in the post-transplant period because of their immunosuppressed state. Many transplant centers are now augmenting early immunosuppression with antibody based immunosuppression-induction. This study is a retrospective analysis of cancer incidence in 1685 liver transplant patients who all received immunosuppression induction with rabbit antithymocyte globulin over a 15 year period.

Methods:

A thorough review of the electronic medical record was conducted for all patients. Any diagnosis of cancer required clinical documentation. Cox regression analysis was employed to assess long term patient survival. Patients with hepatocellular carcinoma (HCC) were analyzed separately from those patients without a history of HCC.

Results:

There were 1685 liver transplant patients included in this analysis with mean follow up of 75 months. There were 10% of patients who had any history of non-HCC cancer at the time of transplant. Among all patients, 16% developed de novo non-HCC post-transplant cancer. Among patients with HCC at transplant, 10% had any history of non-HCC cancer at the time of transplant, with a 15% incidence of de novo cancer post-transplant. Risk factors for post-transplant non-HCC cancer include White race (18%, $p<0.01$), older age (23% for age 60 and older, $p<0.001$), history of alcoholic liver disease (19%, $p=0.03$), and smoking (19%, $p=0.02$). There was an increased risk with increased pack-years (27% for more than 40 pack-years, $p<0.001$). 10-year Cox regression patient survival demonstrates lower survival for patients with any pre- or post-transplant history of cancer, though this does not reach statistical significance.

Conclusion:

These results suggest that patients who develop HCC prior to liver transplant do not have a higher risk of post-transplant de novo non-HCC cancer. There is an increased risk of cancer in these post-transplant patients, but the risk is not higher than that previously reported for solid organ transplant patients who did not receive immunosuppression induction.

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

POSTER #100

INCIDENCE AND MANAGEMENT OF DE NOVO HEAD AND NECK CANCER AFTER LIVER TRANSPLANT AT A SINGLE CENTER IN PATIENTS RECEIVING ANTIBODY-BASED IMMUNOSUPPRESSION INDUCTION

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Introduction:

Solid organ transplant patients are at increased risk of developing de novo cancer in the post-transplant period because of their immunosuppressed state. Many of these patients develop cancers of the head and neck. This study seeks to characterize the incidence and risks factors of post-liver transplant de novo head and neck cancers among 1685 transplant recipients over a 15 year period at a single institution, where all patients received antibody-based induction immunosuppression.

Methods:

The records of 1685 consecutive adult, deceased donor liver transplant (LT) recipients with a minimum 1-year follow-up from 2001 to 2015 were retrospectively reviewed. Incidence of de-novo head and neck cancer was extracted from the original patient population of 1685. There were 121 patients positively identified as having developed de-novo head and neck cancer post-LT. The medical records of these 121 patients were analyzed to determine demographics, history of cancer pre-LT, de-novo cancer type and location, treatment modalities, and alcohol and tobacco exposure prior to LT.

Results:

Of the 121 patients who developed cancer of the head and neck (7.5%), there were 103 cutaneous (6.1%) and 25 non-cutaneous (1.4%). For non-cutaneous cancers, factors associated with increased risk of cancer included alcohol abuse ($p<0.001$), any smoking history ($p=0.02$), and increasing exposure to tobacco ($p=0.02$). The risk of cutaneous cancer was not associated with alcohol abuse or any smoking history, but did increase with greater than 20 pack-years of smoking ($p<0.01$). Cutaneous cancer was also associated with male gender, White race, increasing age and lower severity of liver disease at transplant. A full spectrum of therapies was employed in treatment including surgical excision, radiation and cryotherapy, and chemotherapy.

Conclusion:

The incidence and pattern of head and neck cancer in this population of liver transplant patients was similar to those published previously, suggesting that induction immunosuppression does not increase risk of these types of cancers. Patients with a history of any cancer before liver transplant are not at increased risk for head and neck cancer post-liver transplant.

POSTER #101

BEDSIDE PEWS IS ASSOCIATED WITH THE NEED FOR CRITICAL CARE IN THE PEDIATRIC ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT POPULATION

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Background: Timely identification of a deteriorating pediatric patient can improve outcomes and mortality. While there are scoring systems to identify general pediatric patients in need of critical care, little data exist to support those in high risk patient populations such as the pediatric hematopoietic stem cell transplant (HCT) recipient. The Bedside PEWS has been validated for identifying general pediatric patients at risk of cardiopulmonary arrest.

Objective: We hypothesize that a Bedside PEWS score of = 8 is associated with the need for critical care in the pediatric allogeneic HCT recipient. We further hypothesized that the addition of increased percent weight gain to the PEWS score will strengthen the prediction for the need for critical care.

Design/Methods: Retrospective cohort study of pediatric allogeneic HCT patients from 2009 to 2016. Daily PEWS scores and weights were collected during the transplant hospitalization. PEWS scores prior to PICU admission were compared to max PEWS scores for those who never required PICU. Sensitivity, specificity, positive and negative predictive values were calculated for a PEWS score of = 8.

Results: Of the 102 pediatric allogeneic HCT patients included in the study, 29 were admitted to the PICU. There was no difference in age, sex, ethnicity or diagnosis. There were transplant related variables and complications that were more common in the group that required PICU admission. Table 1. Patients with PICU admissions had a higher mean PEWS score prior to PICU admission than the max PEWS of patients that never required PICU admission (10.4 ± 3.7 vs 4.6 ± 2.3, $p < 0.0001$). A PEWS score =8 had a sensitivity of 79% (95% CI 60 - 92) and a specificity of 90% (95% CI 81 - 96). There was a high negative predictive value at this PEWS score of 92% (95% CI 84 – 96). The addition of a 10% increase in weight gain to a PEWS score of = 8 increased the specificity to 95%.

Conclusion(s): In this study a PEWS score = 8 was associated with PICU admission, having a moderately high sensitivity and high specificity. This study adds to the literature supporting Bedside PEWS monitoring for the HCT patient. While this data supports the use of the PEWS in this population, further research is needed to determine if this scoring system could be further tailored in regards to unique characteristics of this population. Incorporation of weight gain into this scoring system may strengthen its association with the need for critical care.

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

POSTER #102

INHIBITION OF CARBONIC ANHYDRASE IX AS A NOVEL STRATEGY TO ENHANCE THERAPEUTIC TARGETING OF FLT3/ITD MUTATED AML CELLS UNDER HYPOXIC CONDITIONS.

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Background: Recent data suggests that the hypoxic properties of the bone marrow niche play a key role in the evolution of drug resistance in FLT3/ITD+AML, thus indicating a role for hypoxia-specific drugs in AML therapy. However, the mechanisms by which low oxygen (O2) levels distort drug responses of FLT3/ITD+AML cells are mostly unknown and hypoxia targeted approaches are lacking. Here we investigated the cytotoxic activity of classic and novel agents under hypoxic (1%) and normoxic (21%) conditions against FLT3/ITD+AML. **Methods:** Molm14 (M14) cells and primary cells from relapsed/refractory FLT3/ITD+ AML patients were incubated in culture medium under 21% and 1% O2. Cytarabine (Cy) or Quizartinib (Quiz) were added as single agents at the indicated concentrations. After 48h, proliferation and apoptosis induction were assessed per MTT and FACS assays. Cells were also assessed for FLT3 protein and Hypoxia inducible factor (HIF) target gene expression by western blotting and PCR arrays. **Results:** M14 were significantly less sensitive to the cytotoxic effects of Cy and Quiz under 1% O2 compared to 21% O2. Consistently, induction of apoptosis was less under hypoxia compared to normoxia. These results were extended to M13 cells, another FLT3/ITD+ cell line, as well as primary cells derived from patients with relapsed/refractory FLT3/ITD+ AML. Importantly, Quiz appeared to be similarly effective in inactivating FLT3 activity under low and high O2 levels. Therefore, identifying tractable pro-survival genes induced by the low O2 microenvironment should represent a viable strategy to increase the effectiveness of anti-leukemic agents. We next surveyed the effects of Quiz and Cy on a panel of 84 hypoxia-regulated genes using PCR arrays. Our preliminary studies revealed that while the HIF pathway remains largely functional in the presence of Quiz or Cy, these agents blunt induction of several hypoxia genes (incl. CA-IX) in both primary cells and M14 cells. Since CA-IX protein is present in extremely low amounts in only a few normal tissues, CA-IX inhibition may show relatively few side effects compared to standard anticancer drugs. We therefore tested the anti-leukemic activity of FC531 under 21% and 1% O2 and found that this compound confers significant, hypoxia-specific growth inhibition of M14 cells. While not reaching the significance threshold, the trend was similar for the apoptosis promoting effects of FC531. **Conclusions:** 1) The cytotoxic effects of Cy and Quiz are significantly blunted under hypoxic conditions. 2) The HIF signaling pathway remains largely functional under hypoxic conditions, despite treatment. 3) FC531 confers anti-leukemic activity that appears to be confined to the hypoxic niche in FLT3/ITD+ cells. 4) Combinational approaches of anti-AML agents with FC531 are warranted and likely to be effective under micro-environmental stress conditions (e.g. severe hypoxia), thus addressing an unmet need in AML therapy.

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

POSTER #103

CAMKK2 INHIBITION AS A "DUAL-HIT" STRATEGY AGAINST ADT-INDUCED OSTEOPOROSIS AND BONE-METASTATIC PROSTATE CANCER

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Androgen deprivation therapy (ADT), as the standard treatment for prostate cancer (PCa), contributes to significant decreases in bone mass, predisposing the patient to increased fracture risk and a diminished quality of life. Established anti-resorption therapies though effective do not stimulate new bone growth. Hence, there is a critical need for anabolic therapies that reverses treatment-induced bone loss in patients.

Recently, Ca^{2+}/calmodulin-dependent protein kinase kinase 2 (CaMKK2) was shown to play a role in both the anabolic and catabolic pathways of bone remodeling. Pharmacological inhibition of CaMKK2 using STO-609 protects against in ovariectomy-mediated and age-associated bone loss in mice. Further, whereas CaMKK2 is not expressed in the normal prostate, it is highly over-expressed in prostate cancer and thought to be regulated by the androgen receptor. *In vitro* studies have shown that its inhibition suppresses the growth and migration of PCa cells. We observed that CaMKK2 inhibition suppresses the size of 3-D spheroids formed by the androgen-independent cell line C4-2 but not those of the androgen-dependent cell line PC3. However, the exact downstream mechanism by which CaMKK2 regulates PCa growth remains unknown.

Based on these preliminary data, we hypothesize that inhibition of a single target, CaMKK2, will result in the therapeutic alleviation of two chief complications in advanced-stage PCa, i.e., bone metastatic tumor burden and ADT-induced bone loss. To replicate ADT-induced bone loss we performed sham or bilateral orchiectomy (ORX) on pretreated (saline/STO-609) 5-week-old male athymic nude mice (n=15 per cohort). Tri-weekly intraperitoneal (i.p.) injections were continued for 9 weeks (saline/STO-609). Micro-computed tomography analyses indicated a prevention of ORX-induced bone loss in STO-609 treated mice compared to saline treated controls (3-fold, $p<0.05$). Additionally, two weeks after surgery, sham and ORX mice were intra-tibially injected with C4-2B cells. Radiographic and histomorphomteric analyses reveal a decrease in C4-2B-initiated bone lesions in STO-609 treated mice compared to the saline treated cohorts. Taken together, our studies represent a highly novel and unique approach in the treatment of advanced-stage PCa.

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

POSTER #104

NOVEL ANTIBODY-DRUG CONJUGATES FOR PERSONALIZED THERAPY OF LETHAL PROSTATE CANCER

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Treating prostate cancer remains a major unmet clinical challenge despite tremendous preclinical and clinical efforts. Novel therapeutics are critically needed to benefit patients with advanced lethal prostate cancer. We recently identified that the cell membrane protein P-Cadherin is specifically upregulated in prostate tumors that carry deletion of APC, suggesting P-Cadherin as a promising new target for antibody-drug conjugate (ADC) approach. We developed a double cyclic peptide-based linker technology with the goal of building ADCs with enhanced biocompatibility and superior stability. To enable personalized therapy for lethal prostate cancer, we propose to synthesize two new ADC drugs engineered to kill P-Cadherin positive prostate cancer cells selectively. We will demonstrate drug tolerability and anti-tumor efficacy in both cell lines and animal models. We will also explore the synergistic efficacy of combining ADCs with immunotherapy to treat resistant prostate cancer. Our results will provide evidence regarding a novel clinical path for the treatment of advanced prostate cancer.

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

POSTER #105

EFFECT OF CHEMOTHERAPY ON SURVIVAL OF PATIENTS WITH RECURRENT PANCREATIC DUCTAL ADENOCARCINOMA (PDAC) FOLLOWING SURGICAL THERAPY

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Background: Patients with recurrent PDAC are treated similarly to patients with de novo metastatic disease. The aim of this study is to report the outcome of patients with recurrent PDAC following initial definitive resection and adjuvant therapy.

Methods: Patients were identified from an IRB approved, retrospective database at Indiana University that contained patient and tumor characteristics, adjuvant therapy and all treatment for metastatic disease. Follow-up was updated as of 6/2014. Overall survival (OS) from recurrence until death or last follow up was estimated using the Kaplan Meier method.

Results: Between 2008-2014, 451 patients with resectable PDAC and available follow up data were identified, of whom 234 had documented relapse. Patient/tumor characteristics were as follows: median age: 64.2 years (25-89), 54% male, 76% LN+, 24% positive margins, 66% and 85% with lymphovascular and perineural invasion respectively. 69% of patients received adjuvant gemcitabine (GEM). Median time to relapse was 10 months. 60% of patients received at least 1 line of systemic therapy and 18% received 2 lines. Chemotherapy was GEM-based: 33%, 5FU-based 33%, or both 33% and 1% other treatment. Median OS from time of relapse was significantly improved following chemotherapy compared to no chemotherapy (10 months vs 3 months, $P < 0.0001$). Median OS for 5FU based and GEM based treatment was 6 and 8 months, respectively ($P=0.09$). Those who received both had mOS of 14 months (95% C.I 10-17months, $P<0.0001$). On univariate analysis, chemotherapy, regardless of type was associated with a survival benefit.

Conclusion: Chemotherapy appears to be associated with survival benefit for patients with relapsed PDAC following initial curative therapy. Survival in our retrospective study appears similar to reported literature for de novo metastatic disease.

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

POSTER #106

MYASTHENIA GRAVIS IN THYMIC EPITHELIAL TUMORS: INCIDENCE AND PROGNOSIS

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Background: The prognostic effect of Myasthenia Gravis (MG) is controversial. In contrast to traditional belief of negative effect, a recent study from Chinese Database reported superior survival for patients with MG. The aim of this study was to study significance of MG presentation overall survival and compare to that of No MG (NMG).

Methods: This is a retrospective analysis of patients from our institution between1975 to 2015. Patients with a minimum follow-up of 24 months were eligible. Factors analyzed for survival significance include patient factors (age, gender, race) and tumor factors (tumor size, histology grade, Masaoka stage), and surgical resection status. Survival was estimated from the time of diagnosis to the last contact.

Results: A total of 733 consecutive patients were identified from the database: 130 (17.7%) patients with MG at diagnosis (24.0%-126/523 for thymoma, 1.9%~4/210 for thymic carcinoma). MG was a significant factor for overall survival (p=0.011), favoring MG (HR=0.64). The median survivals, 5-/10-year overall survival rates were 192 months (95% CI 148 -244) and 90.4% (95%CI 84.9-96.3)/71.1% (95%CI 61.7-82.0), and 131 months (95%CI 111-164), 75.4% (95%CI 71.1-80.0), 51.1% (95%CI 45.1-58.0), for MG and NMG groups, respectively. There were significant differences in age (p=1.75e-5), gender (p=0.048), stage (p=9.066e-06) and WHO grade of tumor (p= 4.041e-10), no significant difference in race between MG and NMG groups.

Conclusions: MG is associated with superior survival in patients with thymic epithelial tumors. This is similar to results of a modern series from China, differs from our traditional belief. Future study will study whether treatment response of MG is associated with survival.

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

POSTER #107

EARLY ENGRAFTMENT KINETICS (EEK) FOLLOWING NONMYELOABLATIVE ALLOGENEIC PERIPHERAL BLOOD HEMATOPOIETIC CELL TRANSPLANTATION IN HUMANS

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Introduction - Initial immune cell activation and proliferation are critical to the control of viral infections in humans; the cellular arms of the innate and adaptive immunological systems are also the predominant host defense against cancer cells. Much is known about the early events in the control of viral infections in people, but little is known of the early kinetics of the lymphocytic allogeneic attack against hematological cancers. Nonmyeloablative allogeneic transplantation (NMAT) using minimal intensity cyclophosphamide and fludarabine (Childs R, et al, NIH) provided minimal cytotoxic, but highly immunosuppressive conditioning that permitted the timely engraftment of monocytes and neutrophils. The method is characterized initially by mixed and then full donor chimerism and cells are accessible in the blood of recipients in the first week after infusion of the allograft. We hypothesized that the kinetics of donor lymphocytes could be characterized in recipients in the very early post transplant period.

Objectives - Statistically analyze and visually describe the patterns of white blood cell engraftment including the total white blood cell (WBC), neutrophil, lymphocyte and monocyte counts and percentages during the first month of patients receiving hematopoietic cell transplantation (HCT) at the IU Simon Cancer Center from 2010-2016.

Methods - One hundred and twenty two patients with hematological malignancies (median age 52, 59 male 63 female) received either Cy/Flu NMAT conditioning or myeloablative (MAT) thiotepa/Cy conditioning. Total white blood cell counts along with absolute numbers of neutrophils, lymphocytes and monocytes were collected for the first 7 days prior to BMT (Day -7) and the following 22 days post transplant (Day +22). MATLAB 2015a (Mathworks) was used for all statistical analysis and figure creation. Correlation coefficients, T-tests, and survival curves were computed using built-in MATLAB statistical toolbox functions.

Results - The absolute neutrophil counts (ANC) increased above 500/cumm on day +15. Monocytes had a nadir of day -1 and peak at day 15. There was also seen a nadir of lymphocytes day 0 with increasing numbers until day +20. Statistically significant differences ($p<0.05$) were observed for neutrophils from day +10 to day +19, concentrations of monocytes from day +11 to day +13 and percentages of lymphocytes from day +10 to day +19 between the two groups.

Conclusions - NMAT produces similar trends in engraftment kinetics when compared to thiotepa cyclophosphamide conditioning, which differs compared to results of NMAT versus classical myeloablative regimens (TBI/CY; Bu/CY). Several statistically significant differences between the study groups were observed, including the number of days of detectable lymphocytes and a slightly slower monocyte and neutrophil recovery in the NMAT group. These data were useful in helping us understand early engraftment kinetics after NMAT and are being incorporated into mathematical models of engraftment and outcomes after BMT for myeloid malignancies.

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

POSTER #108

A PHASE 1B STUDY OF NAPABUCASIN (BBI-608) PLUS WEEKLY PACLITAXEL IN PATIENTS WITH ADVANCED THYMOMA AND THYMIC CARCINOMA

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Background: Napabucasin (BBI-608) is a first-in-class cancer stemness inhibitor targeting the STAT3 pathway. Synergistic anti-tumor activity of napabucasin plus paclitaxel was observed in pre-clinical and early clinical testing. The STAT3 pathway is considered important in thymic carcinoma and thymoma which are rare cancers with few treatment options. In first line, the objective response rate [ORR], partial response [PR] + complete response [CR] (per RECIST) with carboplatin-paclitaxel was 22% in thymic carcinoma and 43% in thymoma (Gemma, 2011). A phase 1b cohort was established to evaluate safety and preliminary signs of activity of napabucasin plus paclitaxel in these patients.

Methods: Patients with previously-treated advanced thymoma or thymic carcinoma were enrolled with napabucasin (240 - 480 mg orally twice daily) plus paclitaxel (80 mg/m^2 IV weekly for 3 of every 4 weeks). Adverse events were evaluated using CTCAE v4 and tumor assessments were obtained every 8 wks per RECIST 1.1.

Results: A total of 9 patients (thymic carcinoma = 5, thymoma = 4) with a median 3 prior lines of systemic therapy were enrolled.

In thymic carcinoma, the starting napabucasin dose was 480 mg BID (n=2), and 240 mg BID (n=3). Treatment was well tolerated and 1 pt required dose-reduction. There were no grade 3 adverse events reported. As of data cut-off, 3 pts are off-study with progression and 2 remain on treatment. PRs were observed in 4 of 5 patients (ORR = 80%) and the median time on treatment is >7.0 months.

In thymoma, 4 patients received napabucasin 240 mg BID. Adverse events included grade 3 diarrhea and dehydration in 1 patient. As of data cut-off, 1 patient was off-study with progression, 2 due to death not believed secondary to drug (perforated bowel; autoimmune myocarditis), and 1 patient remains on treatment. PR was observed in 1 patient (ORR 25%).

Conclusions: Napabucasin plus weekly paclitaxel has demonstrated an acceptable clinical safety profile and encouraging signs of anti-tumor activity in patients with advanced thymic carcinoma and thymoma. Further clinical evaluation of the combination regimen is warranted in this population.

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

POSTER #109

IDENTIFICATION OF NOVEL PROGNOSTIC FACTORS VIA VOLUMETRIC ANALYSIS IN PATIENTS WITH HEPATOCELLULAR CARCINOMA TREATED WITH LIVER STEREOTACTIC BODY RADIATION THERAPY

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Background

Stereotactic body radiation therapy (SBRT) can cause atrophy of liver tissue in patients with hepatocellular carcinoma (HCC). Untreated liver segments can regenerate to compensate for this loss. We hypothesize that HCC patients with stable or increased untreated liver volume (LV) after SBRT would have improved survival.

Materials/Methods

The study population included 1) patients enrolled on a prospective phase II institutional study of SBRT in HCC and 2) a validation cohort of patients with HCC identified from our institutional cancer registry treated with SBRT alone over the same period. Patients were included from group 1 if they had no evidence of tumor progression during follow-up, a triple-phase CT at 6 mos post-SBRT, and no liver transplant or other liver directed therapy. Patient livers at baseline and 6 mos were contoured and segmented based on venous anatomy. Segment(s) were considered as treated if the PTV extended into that segment; all other segments were considered untreated. Patients were included in the validation cohort if they had a retrievable SBRT plan with complete volumetric data and no other local liver treatment. Univariate analysis (UVA) and multivariate analysis (MVA) were performed using Cox regression. Overall survival (OS) was compared in patients with stable or increased untreated LV after SBRT vs those with untreated LV loss of >5% using Kaplan-Meier analysis.

Results

19 of the 77 patients in the phase II trial met selection criteria. Mean LV/untreated segment volume was 1705 cc (range 987-2627)/1183 (range 714-1987) at baseline and 1551 cc (range 759-2670)/1143 (range 623-1945) at 6 months. Change in volume of untreated segments (*p=0.018)*, liver:GTV ratio *(p=0.064),* and age (*p=0.018)* were significantly associated with OS on UVA and MVA (*p=0.01, 0.021, and 0.015*). Other variables were not significantly associated with OS: baseline Child Pugh class, AFP, GTV, PTV, baseline LV, baseline untreated LV, LV:PTV ratio, and change in total LV (*p>0.1*). 10 patients had LV loss in the untreated segments of >5% and had significantly shorter OS than those without significant LV loss (median OS 12.1 mos vs 97.0 mos, *p=0.011*).

In the validation group, 89 patients met criteria. After adjusting for GTV and PTV, liver:GTV and liver:PTV ratios at baseline trended toward significance on MVA (p=0.064 and 0.076). Patients with liver:GTV ratio >60.8 (the median ratio) had significantly higher OS than those with ratios <60.8 (median OS 71.4 vs 29.0 mos, p=0.025).

Discussion:

LV reduction over time in the untreated segments was associated with significantly worse OS suggesting adequate hepatic reserve is an important parameter for outcome after SBRT. LV:GTV ratio was associated with survival in two independent cohorts of patients with HCC, suggesting the importance of baseline relative LV. Future volumetric studies may assist in assessing hepatic reserve in HCC patients in order to optimize outcomes.

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

POSTER #110

SINGLE-CELL RNA SEQUENCING AND REVERSE PHASE PROTEIN ARRAYS IDENTIFY NOVEL ROLES AND INTERACTING PARTNERS FOR APE1 IN PANCREATIC DUCTAL ADENOCARCINOMA MICROENVIRONMENT

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Pancreatic ductal adenocarcinoma (PDAC) is the 4th leading cause of cancer-related mortality in the US, and is accompanied by a fibrotic phenotype that contributes to chemotherapeutic resistance. Signaling between cancer-associated fibroblasts (CAFs) and tumor are important in the fibrotic phenotype and metastatic spread. Thus, there is a critical need to understand the signaling within the cells of the tumor microenvironment and how it contributes to progression of the disease and resistance to treatment.

A novel PDAC microenvironment target, Apurinic/apyrimidinic endonuclease/redox factor-1 (APE1/Ref-1 or APE1) is a multifunctional protein involved in repairing DNA damage via endonuclease activity and in redox regulation of transcription factors like HIF-1a, STAT3, NFkB and others. APE1 is essential for cell viability, which prevents generation of a stable APE1-knockout cell line. Furthermore, APE1 siRNA knockdowns are transient, which means techniques like bulk RNA-seq are unable to generate a comprehensive list of genes regulated by APE1.

Therefore, we utilized single-cell RNA sequencing to compare the transcriptomes of siAPE1 and scrambled control cells in low passage patient-derived PDAC cells. 1,950 genes were differentially expressed between siAPE1 knockdown and control cells. Pathway analysis identified numerous clinically relevant pathways influenced by APE1 knockdown including mTOR, mitochondrial dysfunction, and apoptosis signaling pathways.

Additionally, we employed Reverse phase protein arrays (RPPA) to analyze changes in expression levels of proteins involved in cancer-specific pathways in tumor cells and CAFs following APE1 siRNA knockdown. The majority of the 304 proteins tested exhibited differential expression patterns between the tumor cells and CAFs, allowing for identification of tumor-specific APE1 targets as well as proteins potentially involved in tumor-CAF crosstalk.

This study utilizes unbiased, statistics-based approaches to validate well established as well as identify new, putative partners and pathways for APE1 in the PDAC microenvironment. This data also allows us identify novel APE1-targeted combination therapies for PDAC treatment.

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

POSTER #111

EARLY NUTRITION DURING CRITICAL ILLNESS IN PEDIATRIC PATIENTS POST-HEMATOPOIETIC CELL TRANSPLANTATION

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Background:
Malnutrition occurs in 20-47% of critically ill children and is associated with poor outcomes including longer PICU stays, prolonged mechanical ventilation, and increased mortality. Hematopoietic cell transplant (HCT) patients are a high-risk population for poor nutrition. There is little data on how nutrition in pediatric HCT patients affects need for critical care interventions. There is also little data evaluating how we feed critically ill pediatric HCT patients after PICU admission and how it affects outcomes.

Objective:
We hypothesized that patients who did not meet goal nutrition by 72 hours post-admission to the PICU had increased in-hospital mortality and increased need for critical care interventions. We defined critical care interventions as need for mechanical ventilation and/or need for renal replacement therapy. Our primary outcome was in-hospital mortality. Our secondary outcomes were 12-month post-transplant mortality, rates of veno-occlusive disease (VOD), need for renal replacement therapy, and need for mechanical ventilation.

Methods:
We performed a retrospective cohort study of pediatric HCT patients admitted to the PICU at Riley Hospital for Children during a 6-year period (January 1, 2009 through December 31, 2014). Patients were included if <21 years, post-allogeneic HCT, and if admitted to the PICU during their transplant admission. Goal nutrition was defined as the goal kcal/day or goal kcal/kg/day set by the nutritionist in the most recent nutrition note in the electronic medical record. We compared patients who did not meet goal nutrition within 72 hours of PICU admission to those who met goal nutrition by 72 hours.

Results:
28 patients were included. There was no difference in gender, diagnoses, donor source, or admission weight categories (underweight, normal weight, overweight/obese) between groups. We found that 54% (15) did not meet goal nutrition by 72 hours from PICU admission. Those that did not meet goal nutrition by 72 hours had higher rates of in-hospital mortality (67% vs 23%; $p = 0.02$) and higher rates of 12-month post-HCT mortality (80% vs 31%; $p < 0.01$). Those that did not meet goal nutrition by 72 hours also had increased rates of VOD (67% vs 15%; $p < 0.01$). There was no statistical difference in need for renal replacement therapy or need for mechanical ventilation.

Conclusions:
We conclude that there is a trend with poor nutrition in the critically ill pediatric HCT patients and in-hospital and 12-month mortality. We also found an association with poor nutrition and diagnosis of VOD. This leads us to question if we sacrifice nutrition for fluid imbalance in the critically ill pediatric HCT population. Further research is needed to evaluate improved nutrition at admission to the PICU and outcomes. Data

from these nutrition studies may be used to inform a standardized nutrition protocol for critically ill pediatric HCT patients.

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

POSTER #112

TRANSGENIC MUSCLE-SPECIFIC OVEREXPRESSION OF MIR-486 DELAYS MAMMARY TUMOR ONSET BUT IS INSUFFICIENT TO OVERCOME FUNCTIONAL LIMITATIONS ASSOCIATED WITH TUMOR PROGRESSION

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Mir-486 expression is enriched in skeletal muscle, which represents ~40% of body mass. Most critically, Mir-486 is an integral part of a myogenesis signaling network that involves Pax7, MyoD, myostatin, and NF-¿B. Furthermore, reduced levels of Mir-486 in muscle is a major defect in muscular dystrophy and muscle-specific transgenic expression of Mir-486 using muscle creatine kinase promoter can rescue muscular dystrophy phenotype in some animal models. Therefore, cancer-induced systemic effects could involve altered Mir-486 levels in muscle/heart, which creates a condition similar to "muscular dystrophy". Indeed, our previous study demonstrated that mammary tumor-bearing mice have lower skeletal muscle and circulating Mir-486 levels, which is consistent with our observations of lower circulating Mir-486 in breast cancer patients. However, it is unknown whether muscle-specific transgenic expression of Mir-486 ameliorates mammary tumors and prevents tumor-induced functional limitations. Thus, in present study, we employed MMTV-PyMT transgenic mice as an animal model of mammary tumor to characterize tumor progression and associated functional limitations. We found that mammary tumor occurred as soon as 7 weeks post birth date, along with limited function performance such as reduced grip strength and impaired rotarod balance, altered body composition and lower Mir-486 levels in muscle and plasma. Next, male PyMT mice were cross-mated with female transgenic Mir-486 mice and double transgenic female mice were tested. Double transgenic mice had a significant slower tumor occurrence, along with a significant difference in body lean mass and body total water in week 12 -post birth date compared to PyMT mice. However, both PyMT and double transgenic mice showed similar defects in grip strength and rotarod performance compared to wild type mice. No differences were observed in body weight, body fat and body free water between double transgenic mice and PyMT mice. These data suggest that Mir-486 alone is insufficient to overcome mammary tumor-induced systemic effects, particularly in muscle functions and altered body compositions. Further study may be necessary to distinguish mechanistic difference between mammary tumor-induced musculoskeletal defects and muscular dystrophy-associated musculoskeletal defects.

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

POSTER #113

PARANEOPLASTIC SYNDROME AND SURVIVAL IN THYMIC EPITHELIAL TUMORS (TET): THE INDIANA UNIVERSITY EXPERIENCE

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Background: Paraneoplastic syndromes (PNS) are commonly associated with thymic epithelial tumors (TET), especially thymoma. The purpose of this analysis is to examine the clinical impact of PNS in TET.

Methods: Patients with pathologically diagnosed TET at a single institution were reviewed retrospectively. The primary and second endpoints for this study were overall survival (OS) and recurrence rates. Clinical factors included age, gender, race, performance score, histology, WHO classification, Masaoka stage, post-operative status, tumor size and number of positive lymph nodes. Cox proportional hazards model was used to identify significant prognostic factors for OS between different PNS groups.

Results: From 1975 to 2016, 733 patients with TET (thymoma (T) -71%, thymic carcinoma (TC) -26% and neuroendocrine tumor (NET)-3%) were seen at Indiana University. Of these, 203 (28%) had PNS including myasthenia gravis (n = 130), red cell aplasia (n = 20), hypogammaglobulinemia (n = 14), systemic lupus erythematosus (n = 12) or other PNS (n = 64). Among these, 37 (18%) had two or more types of PNS. PNS were seen in 35% (183/523) of T, 9% (16/187) of TC and 15% (3/20) of NET ($p < 0.001$), respectively. Recurrence rates and mortality at 5 year were 8% and 10% in PNS (+) group compared to 13% and 16% in PNS (-) group ($p < 0.05$). Intrathoracic recurrences were more common in PNS (+) patients (89% vs 77%; $p = 0.016$). In both groups, adverse factors for survival included: older age, advanced stage, number of positive lymph nodes and TC histology (all p-values < 0.05). However, post-operative R1/2 status was adverse prognostic factor only in the PNS (-) group ($p = 0.001$).

Conclusions: PNS is common in TETs. Patients with PNS have lower risk of recurrence and mortality compared to patients without PNS, but may have a higher risk of intrathoracic recurrence.

Key words: Thymic epithelial tumor; thymoma, paraneoplastic syndrome; overall survival; recurrence; prognostic factor

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

POSTER #114

CLINICAL DOSE-VOLUME HISTOGRAM ANALYSIS FOR RADIATION-INDUCED PROXIMAL BRONCHIAL TREE TOXICITY IN PATIENTS WITH NON-SMALL CELL LUNG CANCER

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Purpose/Objective(s): To study risk factors for radiation-induced proximal bronchial tree (PBT) toxicity in patients treated with thoracic radiotherapy (RT) for non-small cell lung cancer (NSCLC).

Materials/Methods: A total of 100 patients with medically inoperable/unresectable NSCLC treated with definitive thoracic RT in prospective studies were included in the study. PBT and PBT wall (PBTW) were contoured consistently per RTOG 1106 Atlas. The primary endpoint was any grade of PBT toxicity, including bronchial stricture/obstruction/fistula and atelectasis, which was diagnosed and graded based on clinical and radiographic presentation according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 4.0. Dosimetric parameters including mean dose, maximum doses in 0.03 cc, 0.5 cc and 1.0 cc and volumes receiving doses greater than 5-90 Gy (V5-V90) were extracted for both PBT and PBTW. Equivalent uniform doses (EUD) were generated using a power-law based model (a value = 19). Cox proportional hazards model was used to estimate hazard ratios (HR) with 95% confidence interval (95% CI). Receiver operator characteristic curves were constructed to assess the predictive accuracy of positive factors for PBT toxicity and the areas under the ROC curve (AUC) were calculated.

Results: Of the 100 patients, 73 (73%) received 70 Gy or above, 17 (17%) developed PBT toxicity (Grade 1, 9%; Grade 2, 5% and Grade 4, 3%) after a minimal follow-up of 6 months. The median time interval between treatment initiation and onset of PBT toxicity was 8.4 months. Under univariate analysis, none of clinical factors, such as age, gender, tumor volume, tumor stage, was significantly associated with PBT toxicity. Dosimetric factors including EUD, mean dose, maximum doses, V20-V50, V75 and V80 of PBT/PBTW were all significantly associated with PBT toxicity (all P-values < 0.05). Under multivariate analysis, however, only V75_PBT (P = 0.013, HR = 1.03, 95% CI, 1.00-1.06), EUD_PBT (P = 0.022, HR = 1.06, 95% CI, 1.01-1.12), and EUD_PBTW (P = 0.022, HR = 1.06, 95% CI, 1.06-1.12) remained significant. AUCs of V75_PBT, EUD_PBT and EUD_PBTW were 0.66, 0.70 and 0.71, respectively. The dosimetric thresholds to limit the incidence of PBT toxicity to < 30% were 33%, 77 Gy, 78 Gy for V75_PBT, EUD_PBT and EUD_PBTW, respectively.

Conclusion: V75 of PBT and EUD of PBT or PBTW are important significant dosimetric parameters in predicting PBT toxicity in thoracic RT planning. PBT toxicity may be limited by dosimetric constraints.

Key words: Proximal bronchial tree; Toxicity; Dose-volume histogram; Equivalent uniform dose; Lung cancer.

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

POSTER #115

CORRELATING BETWEEN CYTOKINES AND RISKS OF RADIATION PNEUMONITIS

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Introduction: Radiation pneumonitis (RP) is a dose limiting toxicity after radiation therapy for patients with non-small-cell lung cancer (NSCLC), which may lead to chronic complications including lung fibrosis or pulmonary failure, decreased patients life quality, treatment failure, and even life-threatening symptoms. This study applies omics data analysis, along with patient biomedical information, to identify the correlation between plasma cytokine levels and risk of RP.

Materials and Methods: Total 30 serum cytokines were measured from 147 patients with stages I-III NSCLC, undergoing radiation alone or combined radiation and chemotherapy. The patient information included gender, age, smoking status, radiation doses (EQD2), COPD, cardiovascular disease (CVD), Hypertension, Karnofski performance status (KPS), staging group, and chemotherapy. Radiation pneumonitis (RP) was diagnosed and graded according to the NCI's Common Terminology Criteria for Adverse Events. Cytokine measurements were performed in platelet-poor plasma samples at 3 time points: at baseline (within 2 weeks before the start of RT), at week 2, and at week 4 during RT. Platelet poor plasma samples were collected and prepared as previously described. Statistical analysis and data clustering were performed over the data, including multivariate analysis, fisher exact test, logistic regression, and generalized estimating equation (GEE). A predictive model using generalized linear mixed (GLM) model to predict the RP2+ with cytokine expression.

Results: Analysis performed using data from all the three time points (baseline, 2weeks, 4weeks) showed that eotaxin, GMCSF, IL8 and MCP1 had significantly (p-value $< 0.05/21$) lower expression levels in patients with RP2 than without RP2. The clinical significant factors included Gender, RTdose, EQD2, KPS, GTV, Chemo, CVD, and Hypertension. Based on univariate analysis of patient characteristics and cytokine with time, patients' serum levels of eoxatin, IL8, MCP1 at baseline were selected to predict RP2. The most parsimonious GLM model includes IL8 and MCP1 with covariates: RT dose, EQD2, KPS, GTV, Chemotherapy, CVD, and Hypertension. Receiver operative curves (ROC) analysis showed that the predictive model combining baseline MCP1 and IL8 and clinical covariates produced an area under the curve (AUC) of 0.929.

Conclusion: Using cytokines during the whole course of radiation therapy, predictive model using generalized linear mixed model demonstrated excellent predictive values for radiation pneumonitis. Combining cytokines and related clinical factors can predict the potential risk of RP, which is important in guiding the radiation treatment planning and delivery.

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

POSTER #116

FACTORS ASSOCIATED WITH SURVIVAL IN PATIENTS WITH THYMOMA: A STUDY OF 523 CASES FROM ONE SINGLE INSTITUTION

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Objectives:

This study aims to 1) identify factors associated with overall survival in patients with Thymoma from a single Institution, 2) generate a predictive model for survival for better prognostic classification.

Methods:

This is a retrospective study including pathologically confirmed thymoma at Indiana University of from 1975 to 2015. Important factors of patient, tumor and treatment modality were collected from medical records. The staging was carried out according to the Masaoka and SEERs (clinical) staging systems. Overall survival was estimated by the Kaplan-Meier method from the date of the first treatment and prognostic factors were analyzed by a cox proportional hazards regression multivariate analysis. The prediction accuracy of the model was compared using area under curves (AUC) on ROC curves.

Results:

A total of 523 consecutive patients with thymoma were compiled: 53.1% female, 82.2% white. The median age was 50 years (range 13-89). There were 18.7% stage I, 17.0% stage II, 19.5% stage III, 35.4% stage IV and 9.4% unknown. The overall survival rates at 5 and 10 years after diagnosis were 88.7% and 66.0%, respectively. The 5-year survival rates, according to independent significant factors, were as following:

1. Age : <50 years-92.1% and =50 years-85.0% (p= 6.11e-3).
2. Resection status: resected, negative margin-93.5%, resected with positive margin-90.3%, non-resected-84.2% (p=6.34e-3)
3. SEERS stage: local-98.4%, regional-95.0%, distant-76.6%, unknown-89.9% (p= 3.94e-05)
4. Masaoka stage: I-95.8%, II-96.7%, III-90.7%, IV-81.7%, unknown-85.4% (p= 0.0159).

Survival model analysis demonstrated significant improvement in predictive accuracy table below with a model of using all independent factors (age, resection status and stage).

Variates	AUC at 5 years	AUC at 10 years
SEERs stage	0.56	0.56
Masaoka stage	0.57	0.57
Age+ resection status + SEERs stage	0.64	0.64
Age+ resection status + Masaoka stage	0.65	0.64

Conclusions:

Age, resection status, staging groups of SEER and Masaoka are significant factors for overall survival prediction in patients with thymoma. A model of combining age, and stage can predict survival better than

any of the existing staging systems.

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

POSTER #117

PRECISION THERAPEUTIC COMBINATIONS ARE SYNERGISTIC AGAINST TRIPLE NEGATIVE BREAST CANCER USING COMPENSATORY PATHWAYS

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Introduction: Triple negative breast cancer (TNBC) accounts for 15% of all breast cancer cases in the United States, and despite its lower incidence, contributes to a disproportionately higher rate of morbidity and mortality compared to other breast cancer subtypes. In an effort to treat TNBC, a cancer that has no targeted therapies, many have chosen to experiment with combinations of drugs that are chosen for their genomic expression or "hard targets". It has been previously noted however, that single agent therapeutics can change the genomic landscape of both mice and human cells and may be responsible for resistance. Here we show that targeting a compensatory pathway after treatment with a single therapy, results in synergistic combinations and can outperform the choice of hard target therapies.

Methods: Nine TNBC cell lines were chosen based on their abundance of clinically actionable targets. The primary hard target combinations were chosen using DNA sequencing data from TCGA and a board consisting of oncologists and researchers at Indiana University School of Medicine. Compensatory therapies were found using RNA sequencing data from untreated versus single therapeutic treated TNBC cell lines. The merged transcript RPKMs were transformed and analyzed for differential expression. Statistically significant genes were imported into Ingenuity Pathway Analysis (IPA) to identify either therapeutics or genomic targets using the Causal network analysis and Upstream regulator functions. All drug combinations were tested in their respective cell lines and cell viability was assessed via Celltiter-Fluor. Synergy of the combinations was calculated using the Chou-Talalay method.

Results: Using genomically chosen hard targets for drug combinations, all nine cell lines displayed additive or antagonistic results except low nanomolar doses for the MDA-MB-231 cell line. Dosing of MDA-MB-231s with Debrafinib and Pazopanib however, turned highly antagonistic as dosing increased. Using next-generation RNA sequencing data of TNBCs, IPA analysis identified several compensatory targets for each cell line when treated with one of the primary genomically driven drug at its IC50. Using dose escalation of the new drugs with a single hard target drug, we found that each compensatory combination displayed a striking increase in synergy across all TNBC cell lines treated when compared to their original hard target combination.

Conclusion: RNA sequencing of TNBC cells lines treated with single therapies chosen by actionable genomic landscape has revealed compensatory pathways, indicating further druggable targets. These compensatory pathways have been observed to be vital in efficiently treating TNBC cell lines. Using therapeutics that are either FDA approved or in clinical trials we have found that each combination shows strong synergy across all experiments and at lower doses. These data show that choosing a secondary therapy based on compensatory pathways may outperform hard target combinations in the clinic.

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

POSTER #118

TESTICULAR PLASMACYTOMA: A DIAGNOSTIC CHALLENGE.

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Extramedullary plasmacytoma (EMP) is a rare complication affecting 6% of all plasma cell neoplasms. EMP can occur as an isolate presentation, but is more commonly associated with multiple myeloma (MM). Testicular EMP is rare; accounting for only 1.3% of EMP and 0.03-0.15% of all testicular tumors. We present a case of testicular plasmacytoma, misdiagnosed as orchitis, to increase physician awareness to this rare presentation and difficult diagnosis. A 70 year old male with history of MM in remission after undergoing CyBorD followed by autologous stem cell transplantation and maintenance immunotherapy with lenalidomide presented with swelling of right testis for 2 weeks. He reported no pain at the testis and normal urination. Oral quinolone was prescribed with no response. Urinalysis and all cultures were negative. Testicular ultrasound yielded hypoechoic signal of the testis. Biochemical tests showed no evidence of systemic relapse of myeloma. Due to the immunocompromised state, the presumed diagnosis was orchitis. He was treated with prolonged course of intravenous antibiotics for 3 weeks. The lack of clinical response led to biochemical marker screen for germ cell tumors, all of which were negative. Patient underwent orchiectomy, from which tissue diagnosis confirmed plasmacytoma based on positive CD138 and kappa light chain staining. While recovering, patient underwent PET/CT scanning which noted pelvic and retroperitoneal adenopathy with hypermetabolic signal. Fluorescent in situ hybridization (FISH) of the testicular tissue showed t(11;14)(q13;q32), which was not present in the FISH analysis of bone marrow.

This case illustrates the difficulties in diagnosing testicular plasmacytoma, as imaging studies such as ultrasound or CT scans are not specific. The main differential diagnoses of isolated testis enlargement are infections and tumors. While infectious etiology is more common, the negative microbiology work up and lack of response to antibiotics are clues to a non-infectious course. Tissue pathology is the gold standard for diagnosis of testicular tumors. Fine needle aspirate should be avoided though, as skin seeding can occur in cases of germ cell tumors and there is risk of false negatives in the case of lymphoma or testicular plasmacytoma. Transinguinal orchiectomy is the most recommended surgical approach.

EMP represent a sub-clone of bone marrow-independent tumor cells. Therefore, EMP can develop despite systemic remission of myeloma, as presented in our case. Blood-testis barrier may limit tissue penetration of chemotherapy and testes represent a sanctuary site for relapse. t(11;14)(q13;q32) in plasmacytoma has been shown to carry poor survival (Shin HJ et al 2015). While systemic involvement of myeloma is through hematologic spreading, testicular plasmacytoma may follow the pattern of other testis cancers and lymphoma, via lymphatic spreading. Systemic therapy is required once systemic involvement occurs. This patient was treated with pomalidomide, dexamethasone and daratumumab combination (Chari et al 2015), with good response.

Translational/Clinical Research ***Resident physician***

POSTER #119

ANALYSIS OF TOPO II AND P53 BY IMMUNOHISTOCHEMISTRY AND QPCR IN SARCOMA PATIENTS WITH CHEMOFX® ASSAY TO DETERMINE SENSITIVITY AGAINST ADRIAMYCIN AND ETOPOSIDE

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Sarcomas are a heterogeneous group of tumors that account for about 200,000 cancers diagnosed each year and represent 15% of all pediatric malignant tumors. They comprise over fifty subtypes that include bone and soft tissue sarcomas. Bone sarcomas represent about 20% of all sarcomas. It is estimated that over 50% of human sarcomas have a *TP53* mutation. Topoisomerase II (TOPO II) is the target for doxorubicin which is the most effective chemotherapeutic agent, although it is sometimes replaced with etoposide to decrease risk of cardiac toxicity.

In this study, 83 clinical sarcomas were evaluated from the IU Medical Center. Of these 83 clinical cases, 34 were primary tumors, 46 were metastatic tumors. Subtypes included in this study are: Liposarcoma, Leiomyosarcoma, Fibrosarcoma, Malignant Fibrous Histiosarcoma (MFH), Synovial Sarcoma, Osteosarcoma, and Ewings. Paraffin-embedded tissue blocks were obtained from the Indiana University Heath Pathology Laboratory under IRB approved protocols. Immunostainings with TOPOII and P53 antibodies (DAKO) were preformed using the DAKO platform with LSAB2 system at the IU Health Pathology Laboratory. The stained tissues were scanned using Aperio Imaging System and analyzed using the positive-pixel algorithm. In addition, qPCR (TaqMan® assays) was performed using the RNA isolated from the same specimens to analyze TOPO II by a second technique. Live tissue testing was also employed using ChemFX assay was performed on fresh specimens to determine Adriamycin and Etoposide (VP-16) sensitivity and/or resistance. Response to Adriamycin and Etoposide were combined to form a composite precision value and then compared with Clinical Response. This showed a 100% positive predictive value and 90% negative predictive value. We combined the two together to create an overall "Combined Response" Variable. This variable was comparted to Immunohistochemistry for both TOPO II and P53, and the expression levels of TOPOII RNA.

The results showed that the lung metastases expressed a higher level of TOPO II and P53 then did the primary tumors. qPCR and immunohistochemistry data did not correlate with each other. The positive pixel data in the primary tumors were 5.238% for TOPO II and 2.4818% for P53, compared to the metastatic lung lesions with 9.64% for TOPO II and 4.5692% for P53. The combined response versus the categorized TOPO II IHC (into categories of increasing intensity, 1,2,3) had a Chi-squared p-value of 0.656. The combined response to versus the categorized P53 IHC (<5% and >=5%) had a Fisher-Exact test p-value of 0.082. There were approximately 40 resistant and 16 sensitive combined responses available for comparison.

We conclude that P53 is a negative predictor for clinical response, with 12 of 40 resistance cases stained positive for P53, whereas 15 of the 16 sensitive cases were negative for P53.

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

POSTER #120

KIF14 OVEREXPRESSION ON TUMOR FORMATION IN MICE IN A LIFESPAN STUDY

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KIF14 is a mitotic kinesin and microtubule-associated molecular motor that plays an essential role in the last stages of cytokinesis. In multiple cancer types, *KIF14*, overexpression in tumors correlates with stage, aggressiveness, and poor patient outcomes. There is considerable interest in *KIF14* as a possible oncogene, since the *KIF14* locus is in a common region of genomic gain in multiple cancers.

In this study, wild type *BDF*-1 mice and a strain constitutively overexpressing *Kif14*, known as *Kif14*-Tg mice, were evaluated at the end of their natural lifespan, specifically for the occurrence of tumors. There were approximately sixty mice per genotype, and eighty of these mice have died. These mice were necropsied and the following tissues were collected: kidney, liver, lung, spleen, seminal vesicles, ovary, mammary gland, bone marrow, pituitary gland, eye, and brain. Any gross nodules were also collected. These tissues were then fixed in 10% neutral buffered formalin, processed, sectioned, and stained with hematoxylin and eosin.

Although there was no significant difference in mouse weights through the lifespan or in the survival curve between the *Kif14*-Tg and their wild type littermates, a significant increase in follicular lymphoma and diffuse large B cell lymphoma was seen in *Kif14*-Tg mice compared to the wild type mice. Fifty-three mice developed either a follicular lymphoma or a diffuse large B cell lymphoma, and thirty of those mice were *Kif14*-Tg mice. These were the two most common tumors in *Kif14*-Tg mice. Other lesions and tumors that were seen in both strains included thymic lymphoma, sarcomas, myeloid dysplasia, pituitary tumors, and other carcinomas. Non tumorous lesions were seen in both strains of mice including hydronephrosis and telangiectasia in multiple organs. Ballooning degeneration of the lens of the eyes was observed in five mice from the strain *Kif14*-Tg.

Our finding of increased lymphoma and a higher cancer percentage proves the first evidence that *Kif14* can promote tumor formation in a wild-type background. This is the first evidence that *Kif14* may have a role in lymphoma, but complements our earlier finding that *Kif14* overexpression can accelerate tumor formation in a mouse model of retinoblastoma. Together, these outcomes further support *Kif14*'s potential as an oncogene.

Translational/Clinical Research ***Undergraduate student***

POSTER #121

FOLATE TARGETED INTRAOPERATIVE FLUORESCENCE IN LAPAROSCOPIC PARTIAL NEPHRECTOMY

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Background: Evaluating the difference in Folate Receptor a (FRα) expression in normal kidney tissue and renal cell tumors can help differentiate between the parenchyma and the tumor before excision. Coupling the folate analogue OTL38 with the fluorescent tracer indocyaninegreen will allow the local extent of the tumor to be identified, helping with excision with a negative margin and detection of any residual tumor. Due to FRα being 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. In contrast, the renal tumor will not fluoresce and will have low intensity staining. In this study, robot-assisted laparoscopic partial nephrectomy was performed in these patients using FRα immunofluorescence to guide the tumor localization and removal. Immunostaining was used to visualize the tumor and normal adjacent kidney after surgical removal.

Methods: Tissue slides from four patients with renal cell carcinoma (RCC) were evaluated to quantify the difference in FRα expression in parenchyma and tumor. This phase 2 study also evaluated the difference in staining intensity between the normal renal tubules and the tumor cells to confirm the difference in FRα expression.

Results: Three patients demonstrated strong staining of the renal tubules, confirming higher FRα expression in the parenchyma than in the tumor. One patient exhibited high intensity staining in both the renal tubules and the tumor.

Conclusions: Early findings show promise in the use of folate analog OTL38 and a fluorescent tracer as a method of tumor detection and excision. The immunohistochemistry results from 3 patients confirm the difference in FRα expression and staining intensity in renal parenchyma and tumor.

Translational/Clinical Research ***undergraduate student, B.S. in chemistry***

POSTER #122

IN VIVO THERAPEUTICS CORE

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

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

Basic Science ***Core facility***

POSTER #123

CLINICAL PHARMACOLOGY ANALYTICAL CORE

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Basic Science ***Core facility***

POSTER #124

EPIDEMIOLOGY CONSULTATION CORE

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POSTER #125

MULTIPLEX ANALYSIS CORE (MAC)

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POSTER #126

ANGIO BIOCORE

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POSTER #127

KOMEN TISSUE BANK

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POSTER #128

TISSUE PROCUREMENT & DISTRIBUTION CORE

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