



# 2022 Summer Experience Program Abstracts

The University of Texas MD Anderson Cancer Center



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## CPRIT-CURE Summer Undergraduate Program

Abstract Number: 1

### **Role of ATM in Regulating Meiotic Crossovers in Sex Chromosomes and Autosomes**

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**Background:** ATM is a kinase that plays crucial roles in the DNA repair pathway homologous recombination (HR). While multiple pathways for DNA repair exist in somatic cells, meiotic spermatocytes primarily use HR, making spermatocytes an excellent system to study roles of ATM in HR. ATM is essential in limiting the programmed self-inflicted double stranded breaks during meiosis that are required for proper chromosome segregation. Spermatocytes rely on crossovers, products of HR, to connect and segregate homologous chromosomes to generate haploids. Therefore, failure to form crossovers results in aneuploidy, and regulating these DSBs is crucial to maintain genome integrity. Aged mouse spermatocytes have defective crossover formation and thus have higher levels of aneuploidy. Considering that ATM deficiency in spermatocytes result in increased in DSBs, I hypothesize that ATM<sup>+/-</sup> spermatocytes will have increased DSB levels, which will rescue the age-associated loss of crossovers and increase in aneuploidy in spermatocytes.

**Methods:** I used B6xDBA F1 hybrid ATM<sup>+/-</sup> mice and wildtype littermate controls. The testes of adult (2-6m) and aged (18-24m) mice were dissected to generate chromosome spreads. These spreads were either stained with a DNA stain to assess aneuploidy level or stained with antibodies and visualized using immunofluorescence to assess HR repair intermediates.

**Results:** Contrary to our expectations, both adult and aged ATM<sup>+/-</sup> spermatocytes showed similar levels of aneuploidy to that of corresponding WT spermatocyte controls. Sex chromosome aneuploidy decreased while autosome aneuploidy increased in ATM<sup>+/-</sup> as compared to WT spermatocytes. This indicates that decrease in ATM allelic content may rescue age-associated crossover defects specifically in sex chromosomes. To identify the role of ATM in crossover formation, we then analyzed the upstream HR repair intermediate coated with single stranded DNA binding protein RPA. I observed that the number of RPA foci per nucleus in ATM<sup>+/-</sup> aged spermatocytes was reduced as compared to ATM<sup>+/-</sup> adult spermatocytes. This result suggests that the crossover role of ATM may act earlier in the HR repair pathway during the first few steps of the DSB repair.

**Conclusion:** Together, these results provide novel insights on the role of ATM in regulating meiotic crossovers.

**Keywords:** ATM, Aneuploidy, Crossover Regulation, Meiosis

CPRIT-CURE Summer Undergraduate Program

Abstract Number: 2

### **Tumor Microbiome in Murine 4T1 Triple Negative Breast Cancer**

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**Background:** The presence of certain microorganisms has been known to have a strong association with the prognosis and development of human cancers. Recent findings show that the gut microbiome can have a significant role in activating antitumor innate immune responses. In addition, the microbiome in the tumor and its compartment has been found to impact progression and immunotherapy outcomes in patients.

**Methods:** Herein, we utilized 16S deep sequencing platform to profiled 4T1 tumor microbiome of mice treated with immune checkpoint therapy (anti-PD-1 + anti-CTLA4) and found that: 1) 4T1 tumors are mostly colonized by aerobic bacteria, 2) tumor progression may be affected by gut microbiota composition, and 3) immune checkpoint therapy may affect microbiome profile and the biodiversity.

**Results:** Furthermore, preliminary qPCR evidence indicates that 4T1 tumors may feature a low microbial load, compared with pancreatic, liver, and lung tumors.

**Conclusion:** Future applications of probiotics as anti-tumor drug vectors remain open for future exploration.

**Keywords:** Tumor microbiome, ICT, 4T1

CPRIT-CURE Summer Undergraduate Program

Abstract Number: 3

### **Radiation Treatment Reduces Histone Deacetylase Inhibitor Immune Response**

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**Background:** Evidence has shown that the efficacy of radiation therapy is reliant on type 1 interferon signaling and expression promoting an immune response against tumors. This study explored whether HDAC inhibitors could enhance type 1 interferon expression induced by radiation and stimulate the cascade of the cGAS-STING immune pathway to indirectly induce tumor cell death.

**Methods:** Wild-type HEK293 cells and HEK293 LKB1 mutated cells transfected with IFN $\beta$ 1 and A549 lung cancer cells were used in testing the drugs Vorinostat and Belinostat. Luciferase assays were done to measure and image interferon expression in the wild-type HEK293 and HEK293 LKB1 mutated cell types. RT-qPCR was done to measure IFN $\beta$ 1 expression in A549 cells in response to 1  $\mu$ M Vorinostat. A clonogenic survival assay was also done with 1  $\mu$ M Vorinostat to test drug and radiation effects on A549 cell survival.

**Results:** The luciferase assays indicated that radiation had a suppressive effect on immune response by HDAC inhibitors. RT-qPCR indicated a suppression in immune response with the combined Vorinostat and radiation treatment. The survival assay indicated that Vorinostat did not lead to a statistically significant reduction in cell colonies with increasing radiation.

**Conclusion:** Further experimentation can be done to see if other drugs undergo the same effect. Efforts could first be focused on other HDAC inhibitors and seeing if their immune response is also suppressed by radiation. From there, more research would need to be done to figure out the biological mechanism that causes this effect and whether this is reflected in different drug families. In addition, these results could possibly serve as a caution against using certain HDAC inhibitors with radiation to treat cancers such as lung cancer.

**Keywords:** HDAC inhibitor, radiation, immune response, suppression

CPRIT-CURE Summer Undergraduate Program

Abstract Number: 4

### **Impact of OxPho inhibition on pancreatic cancer metabolism and antitumor immunity**

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**Background:** Cancers must shift their metabolic state to support the energy demands of tumorigenesis (1). Cancer cell reliance on upregulated glycolytic and oxidative metabolism depletes oxygen and essential nutrients from the tumor microenvironment, creating a hypoxic, immunosuppressive landscape which limits cytotoxic T cell infiltration (2). In order to offset tumor metabolic advantage, studies have been done utilizing complex I inhibitors of tumor oxidative metabolism to reduce tumor oxygen consumption and combat tumor hypoxia. However, the impacts of inhibition of downstream components of the electron transport chain (ETC) remain poorly understood.

**Methods:** C57BL/6 mice transplanted with MT4-2D pancreatic cells. At day 15 the mice were treated with either IM-156 orally (complex I inhibitor), Atovaquone i.p. (complex II inhibitor) or aTOS i.p. (complex II inhibitor), or Atovaquone orally (complex III inhibitor) for seven days. At the end of the treatment cycle, TILs (tumor infiltrating lymphocytes) and spleen cells were analyzed by high parameter flow using the BDx30. The Seahorse XF Cell Mito Stress Test was used to measure mitochondrial function, oxygen consumption rate (OCR) of MT4-2D tumor cells treated in vitro. Ovalbumin-specific CD8<sup>+</sup> T cells were activated in vitro using peptide and cocultured with increased concentrations of Napyradiomycin (complex I and II inhibitor), aTOS or Atovaquone for 24 hours. T cells were then analyzed by flow cytometry.

**Results:** In this study we found that complex II and complex III inhibition of the ETC reduced the basal respiration of MT4-2D in a dose dependent manner. Inhibition of complex I and II with Napyradiomycin showed a significant decrease in basal respiration and ATP production of MT4-2D. Additionally we saw trends of a possible shift in metabolism from respiration to glycolysis in response to OxPhos inhibitor treatment. Ovalbumin-specific CD8<sup>+</sup> T cells treated with complex II/III inhibitors post activation in vitro showed no significant reduction in viability or activation as seen by Ki67, CD44, and PD-1 staining. MT4-2D tumor bearing mice treated with complex II and complex III inhibitors, respectively, also showed increased CD8<sup>+</sup> T cell function and reduced levels of arginase positive MDSCs and TAMs within the tumor microenvironment.

We show that inhibition of the complex II/III components of the mitochondrial oxidative phosphorylation chain has the potential to offset the tumor metabolic advantage while minimally limiting the activation state of cytotoxic T cells.

**Keywords:** Pancreatic cancer, cancer metabolism, mitochondrial complex inhibitors, cytotoxic T cells

CPRIT-CURE Summer Undergraduate Program

Abstract Number: 5

### **COPD-like Inflammation Induces Neutrophil Invasion and NETosis via the C5a Pathway**

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**Background:** Polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) are a subset of pathologically activated, protumorigenic neutrophils with immunosuppressive activity ranging from enhancing angiogenesis to protecting circulating tumor cells. Recent studies have shown that the ability of PMN-MDSCs to promote tumor cell invasion and metastases may be enhanced by the formation of neutrophil extracellular traps (NETs): weblike structures composed of DNA-histone complexes and other neutrophilic proteins extruded by activated neutrophils. NETs may interact with the tumor microenvironment (TME) to promote tumor cell invasion, proliferation, and metastases through capture of circulating tumor cells and release of regulatory proteins. Stimulatory factors released from the TME – such as neutrophil chemoattractant C5a produced during complement activation – are readily being characterized as major triggers of NET formation (NETosis). However, relatively little is known about the effect of TME-associated inflammation in activating NETosis. In this preliminary study, we predict that COPD-like inflammation promotes NETosis in the lung tissue via a C5a-dependent mechanism.

**Methods:** We generated COPD-like inflammatory conditions in C57BL/6 mice through once weekly administration of aerosolized Nontypeable Haemophilus influenzae (NTHi) lysate from 6 to 14 weeks of age. Frozen tissue, bronchoalveolar lavage fluid (BALF), and whole lung samples were collected from wild type and NTHi-exposed mice. We performed qPCR to measure expression of the C5a/C5aR1 axis and downstream receptors, Sytox staining to quantify NETosis, Wright-Giemsa staining for immune cell lineages, and H&E for visualization of immune cell infiltration.

**Results:** NTHi exposure promotes immune cell invasion, neutrophilic influx, and a higher percentage of NETotic neutrophils. NTHi exposure also upregulates the transcription of C5aR1, Tlr4, and Ager (RAGE) receptors involved in NETosis.

**Conclusion:** Our results indicate that NTHi may activate NETosis through upregulation of the C5a/C5aR1 axis and other PMN-MDSC surface receptors. Future steps include validating our results on the protein level and expanding the number of treatment groups to examine NTHi exposure and NETosis inhibition with DNase in a lung cancer mouse model (CC-LR). We hypothesize that NTHi exposure promotes NETosis, tumor cell invasion, and metastases in lung cancer through the upregulation of downstream C5a signaling present on tumor-associated PMN-MDSCs, and that treatment with NETosis inhibitor DNase will significantly reduce these protumorigenic effects. Confirmation of these results may reveal the potential of NETosis inhibition by DNase as a targeted therapy in the treatment of lung cancer.

**Keywords:** NETosis, NTHi, C5a, lung cancer

CPRIT-CURE Summer Undergraduate Program

Abstract Number: 6

### **Multimodal Analysis of the Interaction Between CD70-Directed Chimeric Antigen Receptor Natural Killer Cells and Target Tumors**

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**Background:** Chimeric antigen receptor natural killer (CAR NK) cells have emerged as an attractive form of cancer immunotherapy. In addition to a promising safety profile—little or no evidence of cytokine release syndrome (CRS), graft-versus-host disease (GvHD) in HLA-mismatched patients, or neurotoxicity in multiple clinical trials—CAR NK cells offer several logistical advantages compared to the current FDA-approved autologous CAR T cell therapies. CD70 is a surface antigen that is highly expressed on multiple tumor types, ranging from solid cancers to hematologic malignancies. Thus, targeting CD70 holds promising therapeutic potential. As such, NK cells transduced to express a CD27-CAR can be used to target CD70-expressing tumor cells. CD27 is a tumor necrosis factor receptor (TNFR) family member and the ligand of CD70. However, the interaction between CD27-CAR NK cells and CD70+ targets has not been thoroughly investigated. This study aims to compare the conjugate formation between CD27-CAR NK cells and nontransduced (NT) NK cells, as well as their cytotoxic capacity and metabolic fitness.

**Methods:** CAR NK cells were obtained by retroviral transduction with a vector encoding a CD27 receptor and containing a CD3 $\zeta$  endodomain. The CAR NK cells were armored with IL-15 to support survival, stimulated with IL-2 for proliferation, and expanded with feeder cells. Cell lines—MM.1S (multiple myeloma), Raji (Burkitt lymphoma), Karpas (non-Hodgkin's lymphoma), K562 (leukemia), UMRC-3 (renal cell carcinoma)—were employed to assess killing function of the CAR NK and NT NK cells. Standard <sup>51</sup>Cr release assay was conducted at various effector-to-target ratios to assess antitumor effect and sibling CAR NK killing. Samples were stained and ran through Amnis imaging flow cytometer (Luminex) and LSRFortessa X-20 Cell Analyzer (BD Biosciences). Flow cytometry analysis was performed using Kaluza Software (Beckman Coulter). The Cell Mito Stress Test and Glycolysis Stress Test were run on 96-well Seahorse XF Extracellular Flux Analyzer (Agilent Technologies) to measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), respectively, of NT and CAR NK cells. Liquid



chromatography-mass spectrometry (LC-MS) was conducted in the MD Anderson Cancer Center Metabolomics Core. Quantification was done within TraceFinder software (ThermoFisher Scientific). The data were analyzed in GraphPad Prism and a normalized heat map was generated in Morpheus (Broad Institute).

Results: CD27 on the CAR NK cell was able to engage CD70 on the tumor cell at the cell-cell interface, also known as the immunological synapse (IS). Upon conjugate formation, CD3 $\zeta$  in the CAR NK—but not the NT NK cell—accumulated at the IS. This passed the activating signal, resulting in greater CAR NK cell-mediated cytotoxicity of CD70-expressing cancer cells. This greater cytotoxic capacity was due to the enhanced metabolic fitness of the CAR NK cells, as evidenced by increased glycolytic and TCA cycle activity. We also found that the CD27-CAR NK cells exhibited minimal CAR NK sibling killing and limited off-tumor on-target toxicity, despite HLA mismatch.

Conclusion: CD27-CAR NK cells exhibit increased metabolic fitness, more immunological synapse formation, and greater cytotoxicity compared to their NT counterparts. The CAR-transduced cells also avoid sibling fratricide, likely due to pre-existing receptor-ligand binding on cis CAR NK cell (also known as masking), or downregulation of CD70.

Keywords: Natural killer (NK) cell, chimeric antigen receptor (CAR), CD27, CD70, conjugate

CPRIT-CURE Summer Undergraduate Program

Abstract Number: 7

**Network analysis of gut microbiome throughout a whole foods based high fiber dietary intervention reveals complex community dynamics in melanoma survivors**

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**Background:** Recent evidence has demonstrated that the gut microbiome modulates response to immune checkpoint blockade (ICB) treatment in melanoma patients. Microbiome modulation via a habitual high-fiber diet was associated with significantly improved progression-free survival (PFS) in melanoma patients on ICB. Previous findings have suggested that this pro-response is associated with known fiber-responsive taxa and Short Chain Fatty Acid (SCFA) producing taxa. However, little is known about the communications responsible for stimulating the aforementioned taxa. To explore community dynamics and identify potential keystone communicating taxa, we conducted microbial association network analysis throughout a high-fiber dietary intervention (HFDI) in melanoma survivors.

**Methods:** Ten patients were enrolled to the HFDI study and were provided with whole-food-based fiber-enriched meals for the duration of six weeks. Fecal samples were collected longitudinally, and whole genome sequencing (WGS) of the fecal microbiome was used to calculate microbiome composition profiles. OTU abundance data was then used to construct, analyze, and compare association networks across timepoints via the R package NetCoMi. Overall changes in community dynamics were assessed via changes in global network properties, and significant taxa were identified quantitatively via differences in calculated centrality measures as well as visually by NetCoMi's selection of hubs, or specific keystone taxa.

**Results:** Network analysis across timepoints demonstrated a general increase in connectivity by both quantitative and visual comparison throughout the HFDI. Analysis via multiple association statistics revealed a general rise in several measures of centrality of many known fiber-responsive and SCFA-producing taxa, with many consistently becoming hub taxa. Consensus networks generated by overlapping several networks generated from different association statistics revealed a consistent increase in centrality in two particular species: *Ruminococcus bromii* and *Dorea longicatena*. Analysis of networks constructed from only differentially associated taxa revealed similar results, with *R. bromii* and *D. longicatena* having numerous changes in associations with fiber-responsive taxa.

**Conclusion:** Increases in general connectivity measures indicate that a HFDI prompts the gut microbiome to become a more interconnected and dynamic community over time. This, in conjunction with an overall increase in centrality in known fiber-responsive and SCFA-producing taxa, reflects an overall pro-response to the HFDI. Networks constructed via different association statistics yield varied results, with many identifying different taxa as hubs. Despite this heterogeneity, network analysis consistently

identified *D. longicatena* and *R. bromii* as potential keystone species with an important role in communicating with fiber-responsive taxa. Further analyses are needed to characterize communications between keystone species and SCFA-producing taxa.

Keywords: microbiome, fiber, networks, melanoma, immunotherapy

CPRIT-CURE Summer Undergraduate Program

Abstract Number: 8

### **Defective Homologous Recombination in Aged Mice**

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Age is one of the biggest risk factors for cancer, a disease which is often characterized by an accumulation of DNA damage. We wanted to test if DNA repair machinery responsible for mitigating DNA damage degrades with age. Homologous recombination (HR) is one of the least error-prone DNA repair pathways and is the primary pathway utilized during meiosis. Thus, we used meiotic homologous recombination as a paradigm for studying age-related degradation of HR machinery. Meiotic cell division requires the formation of crossovers, specific products of HR, to connect homologous chromosomes for accurate segregation. To initiate HR, intentional double-strand breaks (DSBs) are introduced into the chromosomes by an enzyme called SPO11. Meiotic cells lacking enough crossovers fail to form viable embryos in most scenarios. Therefore, the cell works to maintain the required number of crossovers despite fluctuations in DSB numbers in a process termed "Crossover Homeostasis." We tested if crossover homeostasis was disrupted in aged mice.

Male mice heterozygous for the Spo11 null allele were naturally aged for a period of 18-23 months. Testes from aged mice were dissected, spermatocytes were isolated, and utilized for chromosome spreads. Spreads were stained with Giemsa to analyze nuclei in Metaphase. Immunofluorescence staining was performed to count DSBs.

Consistent with earlier reports, decreasing an allelic copy of Spo11 leads to a reduction in DSB formation in both Adult and Old mice. Removing a copy of Spo11 causes crossover loss only in Old mice and not in Adult mice, demonstrating a defect in crossover homeostasis in Old mice. Further a proportional level of aneuploid secondary spermatocytes was observed after the first chromosome segregation, indicating that Metaphase I cells with unconnected chromosomes make it past the first meiotic checkpoint and could produce aneuploid gametes.

We demonstrated that Homologous Recombination is defective during meiosis in old mice. Specifically, we saw that crossover homeostasis is affected. Discounting mutants that directly perturb the crossover repair pathway, this is the first ever observation of a defect in crossover homeostasis in any organism. The mouse model was aged naturally, and the genetic perturbation doesn't affect the DNA repair machinery, thus allowing our observations to become transferrable to old somatic cells and correspondingly old humans (70-90 years old). Considering that this is typically the age when DNA mutational load causes diseases such as cancer, this research becomes relevant for the development of therapeutic interventions to tackle the root causes of such age-related dysfunction.

Keywords: DNA repair; Meiosis; Crossover Homeostasis; Homologous Recombination; SPO11

CPRIT-CURE Summer Undergraduate Program

Abstract Number: 9

### **Dissecting the role of Muc5ac in K-ras Mutant Lung Cancer Progression**

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**Background:** Of all cancer types, lung cancer accounts for the most deaths per year and K-ras mutations are a leading mutational driver. There are limited pharmacological treatment options for K-ras mutant lung cancer due to the resilient nature of the mutation, raising the need to find and target downstream or cooperating effectors of K-ras. The mucosal lining of the airways is integral for airway homeostasis and functions to clear pathogens and debris. In healthy tissue, lung epithelial cells secrete two main mucins: moderate levels of muc5b and low levels of muc5ac. Notably, patients with K-ras mutant lung adenocarcinoma (KM-LUAD) have been shown to commonly overexpress muc5ac. Overexpression of muc5ac can promote respiratory illnesses through its promotion of an inflammatory lung environment, which can lead to promotion of KM-LUAD. Through a genetic approach, we previously observed that knocking out the gene that encodes for MUC5AC (Muc5ac) in a K-ras mutated lung adenocarcinoma mouse model (CC-LR) leads to significantly decreased tumor burden. Therefore, Muc5ac might be a potential druggable target in KM-LUAD which we explored in this study.

**Methods:** To translate our genetic targeting approach and dissect the potential mechanism of tumor promotion by muc5ac, we utilized CC-LR mice and a pharmacologic approach for inhibiting Muc5ac expression: RNA interference (RNAi). CC-LR mice had muc5ac RNAi treatment started at 6 weeks for a treatment duration of 8 weeks. Treatment, both of drug and vehicle, are administered through intratracheal injections. Mice are anesthetized using Isoflurane and a pipette is inserted into the trachea to administer 50 microliters of treatment. MUC5AC RNAi treatment were given twice a week (5mg/kg biweekly).

**Results:** There was a significant reduction of surface tumor numbers with inhibition of Muc5ac by RNAi. Bronchoalveolar lavage fluid (BALF) analysis revealed a trend for increased total white blood cell (WBC) count and number of macrophages after RNAi treatment. Muc5ac knockdown decreased expression of Il6, a cytokine with known pro-tumor function. Muc5ac RNAi also reduced the expression of immunosuppressive macrophage markers including Fizz1 indicating a possible phenotypic switch in macrophages from M2 (pro-tumor) to M1 (anti-tumor). There was a reduction in expression of Siglec-e, a cell surface protein primarily expressed on macrophages, elucidating its potential immunomodulatory role through its interaction with muc5ac.

**Conclusion:** We conclude that targeting Muc5ac using RNAi is a potential alternative approach for prevention and treatment of KM-LUAD via tumor cell intrinsic and immunomodulatory paracrine mechanisms.

**Keywords:** Muc5ac, Lung Adenocarcinoma, K-ras, Siglec-e

CPRIT-CURE Summer Undergraduate Program

CPRIT-CURE Summer Undergraduate Program

Abstract Number: 11

## **The Genomic and Transcriptomic Landscape of Ultra-Conserved miR-142 in Hematological Malignancies**

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**Background:** MicroRNAs (miRNAs) are noncoding RNAs that regulate gene expression by targeting the 3'UTR of mRNAs. Dysregulation of miRNAs are often linked to cancer. Our lab demonstrated that among various miRNAs and cancer types, a high frequency of mutations is concentrated in miR-142 and hematological malignancies, particularly chronic lymphocytic leukemia (CLL).

**Methods:** Targeted deep sequencing of ultra-conserved elements (UCEs) of 348 clinically well-annotated cancers was performed by MD Anderson and collaborating institutions. Sanger sequencing was conducted in the UCE\_5578 region containing miR-142 of 400 CLL (200 indolent; 200 aggressive) patient genomic samples. HEK293 cell lines were transfected with retroviral plasmids expressing UCE\_5578 with miR-142 scrambled, wild-type, or mutated at the 6th nucleotide downstream. This was followed by RNA extraction, reverse transcription, and RT-qPCR to compare expression levels of pre-miR-142, miR-142-3p, and miR-142-5p.

**Results:** We identified 5 mutations within UCE\_5578, a region found to contain miR-142: two in the miR-142-5p seed and one in miR-142-3p. One notable C to T mutation occurred in the 6th nucleotide downstream of the miR-142 sequence at the CNNC motif known for SRSF3 binding and recruitment of DROSHA. Expression of UCE\_5578 with this mutation in HEK293 cells resulted in a downregulation of miR-142-3p levels compared to wild-type expression levels.

**Conclusion:** Our findings suggest that the C to T mutation 6 nucleotides downstream of miR-142 at the CNNC motif impaired miR-142-3p processing in CLL. This could potentially upregulate miR-142-3p targets with links to leukemogenesis. The effect of other mutations identified in CLL will be further investigated.

**Keywords:** Chronic Lymphocytic Leukemia, Ultra-conserved elements, miR-142

CPRIT-CURE Summer Undergraduate Program

Abstract Number: 12

## **Automated Ligand Identification System (ALIS): A Novel Small Molecule Screening System in Cancer Drug Development**

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Small molecule drugs are crucial blockbusters in cancer treatment for their wide availability and simple dosing; however, discovering them using traditional high-throughput systems (HTS) can be extremely costly. The Automated Ligand Identification System (ALIS) is a novel affinity-based binding system that identifies small molecule binders for cancer target Protein X more efficiently than HTS. ALIS's accurate mass analysis and direct protein-ligand binding, as opposed to HTS's protein coupling, results in lower false positive hit rates and experiment trials.

First, buffer conditions for the ALIS screen were determined with a Differential Scanning Fluorimetry (DSF) experiment. DSF monitors thermally induced protein denaturation via use of a dye (Sypro Orange) which binds preferentially to unfolded protein. Dependence on NaCl in buffer was tested by determination of the protein's melting temperature (TM) as it was increased in assay buffer. Concentrations of 11, 50, 100, 200, and 400 mM of NaCl were tested with Protein X. Once optimal NaCl concentrations were determined, the assay buffer was created with ammonium acetate, HEPES, phosphate, and NaCl. Protein X and assay buffer were incubated with compound pools in a 384 well plate for a 16-hour ALIS screen. An experimentally determined switch valve diverted the initial ~80% of protein, along with bound compounds (named "hits"), from the size-exclusion-chromatography column to reverse-phase column, where heat and acid denatured the protein and released bound molecules. These molecules eluted to the mass spectrometer to be ionized and detected.

Protein X showed the greatest stability with >200mM NaCl due to its right shifted melt curve and height of >10,000 fluorescence units. Data is collated from Apex software to determine the frequency of every ion identified. Ions whose frequency is >7 times are contaminants and discarded. Ions that occur one time are "unique" hits and kept. Ions that occur 2-6 times are further examined. For further examination, the total-ion-chromatogram is scanned at each ion's retention time to determine whether the hit is reported in neighboring wells. If so, the hit is most likely a contaminant. The mass ion cluster chromatogram indicates a true hit if its abundance is  $100,000 < x < 1,000,000$  and has a correlation of >0.950.

Using these analyses, the initial 2000 hits were filtered to 50 hits, eliminating false positives and contaminants. Next, ALIS screens between the 50 hits and Protein X will be conducted to verify protein-ligand interactions, measure association and dissociation rates, and binding affinities.

Keywords: drug development, therapeutics, high throughput screening, in-vitro pharmacology

CPRIT-CURE Summer Undergraduate Program



Abstract Number: 13

### **Gut Microbiome Signatures Associated with Liver Tumorigenesis in HepPten- Mice**

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**Background:** The number of patients with non-alcoholic fatty liver disease (NAFLD) and hepatocellular carcinoma (HCC) continues to increase globally in tandem with the obesity epidemic. Individuals with NAFLD-related HCC are typically diagnosed at a late stage and have poor odds of survival. As such, highly sensitive biomarkers and preventative measures are needed to identify individuals at high risk of HCC.

**Methods:** We aimed to characterize the gut microbiome and find taxa associated with the development of HCC in 23 female mice with hepatocyte-deletion of Pten- (HepPten-). Stool samples of 23 female HepPten- mice were collected and analyzed using 16s sequencing. Mice were necropsied at different stages of tumor development. Among all histology parameters analyzed, tumor burden, number of tumors and inflammation, showed significant differences between Partitioning Around Medoid (PAM) clusters. To identify the specific taxa associated with high tumor burden, number of tumors or inflammation scores, LEfSe analyses were performed from kingdom to species.

**Results:** A total of 33 significant taxa were identified associated with high tumor burden ( $>89 \text{ mm}^3$ ). Similarly, 38 taxa were identified to be significantly associated with high number of tumors ( $>2$ ). The gut microbiome of mice with high tumor burden and number was enriched with *Gordonibacter*, *Senegalimassilia*, *Lachnospiraceae\_UCG-006*, *Monoglobus*, and depleted of *Prevotellaceae* and *Bacteroides\_vulgatus*. Thirty exclusive taxa were identified to be significantly associated with higher liver inflammation scores ( $>0.5$ ) by LEfSe analysis. The gut microbiome of mice with high liver inflammation scores was enriched with *Butyrivibrio*, *Oscillibacter*, *Family\_XIII\_UCG-001*, and depleted of *Olsenella\_uli*, *Muribaculaceae\_ge\_unclass*, and *Faecalibaculum\_rodentium*.

**Conclusion:** These results therefore suggest that specific microbiome components are significantly associated with liver carcinogenesis.

**Keywords:** NAFLD, gut microbiome, HCC, HepPten

CPRIT-CURE Summer Undergraduate Program

Abstract Number: 14

### **The Role of hnRNPC::RARG in Acute Promyelocytic Leukemia-like Acute Myeloid Leukemia**

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**Background:** Acute promyelocytic leukemia (APL), a subtype of acute myeloid leukemia (AML), is distinguished by t(15;17)(q24.1;q21.2)/PML::RARA, characteristic morphologic features, and a propensity for disseminated intravascular coagulopathy. The fusion of PML with retinoic acid receptor  $\alpha$  (RARA) gene accounts for response to all-trans retinoic acid (ATRA), allowing APL patients to have a favorable prognosis. However, there have emerged certain cases in which patients who, despite morphologically and clinically resembling APL, were resistant to ATRA. Rather than the PML::RARA, these patients presented with an atypical fusion of retinoic acid receptor  $\gamma$  (RARG) gene, with a myriad of fusion partners. One of these fusion genes involves exon 3 of hnRNPC fused with exons 4-10 of RARG. We aim to create a retroviral vector containing an hnRNPC::RARG insert in order to study the role of hnRNPC::RARG fusion protein in leukemogenesis and develop targeted therapy.

**Methods:** Recombinant cloning was performed to create a retroviral vector (MSCVI) containing an hnRNPC::RARG insert using overlapping PCR, purification, digestion by restriction enzymes, ligation, transformation of DH5-alpha E. Coli, and miniprep. The sequences of the resulting miniprep plasmids were confirmed using Sanger sequencing.

**Results:** A plasmid consisting of an MSCVI backbone, the whole coding sequence (CDS) of hnRNPC, and an HA tag was first constructed. A similar plasmid was constructed separately, except with the CDS of RARG. Sanger sequencing of the plasmids confirmed the correct hnRNPC and RARG sequences, respectively. These two were then used as the templates for creating the hnRNPC::RARG fusion gene, using primers designed to create overhangs at the 3' end of exon 3 of hnRNPC and 5' end of exons 4-10 of RARG that would facilitate fusion during PCR. Sanger sequencing performed over the hnRNPC::RARG fusion confirmed the correct fusion sequence without mutation.

**Conclusion:** We created the hnRNPC::RARG fusion which is one of an unknown myriad of translocations that may drive the transformation of an "APL-like AML". We will produce an hnRNPC::RARG retrovirus via co-transfection of 293T cells using the retroviral vector crafted in this study, along with the packaging genes gag, env, and pol. Further study of this fusion protein may provide great insight into the pathogenesis of APL-like AML and mechanisms of resistance to ATRA, as well as reveal new therapeutic targets.

**Keywords:** APL, RARG, hnRNPC::RARG, recombinant cloning

CPRIT-CURE Summer Undergraduate Program

## CP RTP Summer Research Experience

Abstract Number: 15

### **Cigarette smoking and its impact on the survival outcomes and molecular features of metastatic colorectal cancer patients**

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**Background:** Tobacco use is the most preventable cause of cancer. Smoking itself accounts for approximately 30% of all cancer-related deaths in the United States. Furthermore, colorectal cancer (CRC) is the second most common cause of cancer death in the United States. In 2020, approximately 147,950 individuals were diagnosed with CRC and 53,200 died from the disease. 11%-22% of these new CRC cases were attributable to tobacco use. Although studies have demonstrated a significant association of cigarette smoking with CRC incidence and mortality, there is very little existing literature that has shown that cigarette smoking may be a risk factor for metastatic colorectal cancer (CRC). The impact of smoking on the survival of metastatic CRC patients remains unclear.

**Methods:** With an initial metastatic CRC diagnosis on or after January 1st, 2012, we reviewed 2,324 patients whose clinicopathological background included a smoking history and 3,826 patients whose background did not from the University of Texas MD Anderson Cancer Center medical records. Univariate and multivariate analyses using Kaplan Meier survival curves and the Cox proportional hazard model were performed to determine the impact of independent prognostic variables on the survival of smokers and non-smokers.

**Results:** The aims of this research study were to associate cigarette smoking with survival outcomes and molecular features of metastatic colorectal cancer patients by analyzing an institutional database. Preliminary data suggests that smoking history has a modest effect on overall survival in metastatic patients compared to a never smoker. It also suggests that there may be a correlation between smoking and KRAS/BRAF mutations.

**Conclusion:** Smoking is linked to survival outcomes and molecular features of metastatic colorectal cancer. The association of smoking with colorectal cancer and its clinical, pathological, epidemiological, and molecular features still needs to be better understood. Future research findings will help point to risk factors involved with smoking in colorectal cancer patients and at-risk patients. Further findings may help to identify markers and patterns that will lead to a faster metastatic CRC diagnosis and an overall better prognosis (improved survival rate and increased survival time). Ultimately, the findings should aim to push forward the agenda of smoking cessation in metastatic CRC patients, at-risk patients, and the general population.

**Keywords:** Smoking, metastatic colorectal cancer, survival analysis, survival outcomes, molecular features

CP RTP Summer Research Experience

Abstract Number: 16

### **Sun protection awareness, habits, and recommendations of USA Swimming coaches**

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**Background:** Swimming is the most popular recreational activity for children and teens (7-17) in the United States. The governing body for the sport, USA Swimming has more than 400,000 members and continues to grow rapidly. Despite the sport's growth, there has not been a paralleled growth in skin cancer awareness or prevention strategies. Current recommendations for preventing skin cancer include wearing sun protective clothing, applying sunscreen, and avoiding direct sun exposure. Individuals in the sport of swimming face unique barriers to following these prevention strategies that have yet to be addressed.

**Methods:** This study is a cross-sectional, survey-based study of swimming coaches' personal attitudes and practices of sun protection, what recommendations they make to their athletes and their choice of practice times. The RedCap survey was disseminated via email to 67,000 coaches registered with USA Swimming. Responses were recorded, and the results were analyzed using the statistical software SAS.

**Results:** Preliminary data show that Coaches who have former athlete(s) with a skin cancer diagnosis are more likely to use sun protection than those with personal or family skin cancer histories. Furthermore, coaches with increased sun protection habits are more likely to recommend sunscreen use to their athletes. Finally, our results have shown that coaches who make sun protection recommendations to their athletes observe higher rates of sunscreen use amongst their athletes.

**Conclusion:** The associations identified between skin cancer history, sun protection habits, and the sun protection recommendations USA swimming coaches make to their athletes suggests a need for the development of sun protection strategies unique to the sport of swimming. These strategies should aim to educate coaches on proper sun protection habits and effective, persuasive communication of those habits to their athletes.

**Keywords:** sun protection, sunscreen, swimming, coaches, skin cancer

CP RTP Summer Research Experience

### **Disparities in Health-Related Quality of Life among Adolescent and Young Adult (AYA) Cancer Survivors**

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**Background:** Adolescent and young adult (AYA; individuals diagnosed between the ages of 15–39 years) cancer survivors represent an understudied population. While extant studies highlight the correlation between poor health-related quality of life (HRQoL) and low survival rates in adults, there is limited research on factors that mediate HRQoL in AYAs with cancer. Moreover, a recent study suggests that the burden of poor HRQoL differs by race/ethnicity.

**Methods:** This study consists of a large retrospective analysis of 2,572 AYA cancer survivors (1,737 White, 241 Black, 466 Hispanic, and 128 Asian) from MD Anderson. Patient reported quality of life at diagnosis (+/- 6 months) was assessed using the SF-12 questionnaire with generation of physical component summary (PSC) and mental component summary (MCS) scores. ANOVA was used to assess differences across race/ethnicity for tumor type, age at diagnosis category, and gender. Logistic regression was conducted to determine odds ratios and corresponding 95% confidence intervals for sarcoma tumors, age at diagnosis, and gender for each racial/ethnic group.

**Results:** In older AYAs, there were significant differences in PCS across racial/ethnic groups ( $p=0.02$ ), with older AYAs displaying favorable PCS scores compared to younger AYAs. The risk of a poor PCS was elevated for all younger AYA racial/ethnic populations and reached statistical significance in White AYA cancer survivors (OR:1.27, 95% CI:1.00-1.61). Compared to White sarcoma patients, minority sarcoma AYA patients report poorer PCS scores. Risk of poor PCS was found to be significantly higher for Hispanic survivors (OR:3.06, 95% CI: 1.36-6.87). Generally, while male AYA patients were found to have a higher risk of poor PCS, they also had a lower risk of poor MCS relative to female AYA patients of the same race/ethnicity.

**Conclusion:** Results highlight sarcoma tumors, gender, and age at diagnosis as significant mediators of poor HRQoL by race/ethnicity among 2+ year survivors of AYA cancer. Findings may inform future health promotion efforts to reduce racial and ethnic disparities in health-related quality of life among AYA cancer survivors.

**Keywords:** Health-related quality of life, Short Form-12, Adolescent and Young Adult

CP RTP Summer Research Experience

Abstract Number: 18

### **An Analysis of Post-Radiation Therapy and Clinical Disease Predictors in the Development of Xerostomia and Dysphagia in Head and Neck Cancer Patients**

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Head and neck cancers (HNC) are the sixth most common types of cancers in the world. Current studies suggest that head and neck cancers are highly treatable and preventable, since most HNCs are squamous cell carcinomas. The standard treatment of care involves surgery, chemotherapy, and/or radiation therapy (RT). While surgery alone has been proven effective in targeting and eliminating oropharyngeal carcinomas, surgery induces metabolic stressors, which heighten the incidence of recurrences. So, cancer management through radiotherapy is a core mechanism for minimizing tumorigenesis. A major concern with the use of RT is the unintended targeting of normal soft tissues. RT often produces toxicities, or side effects, which undermine the effectiveness of head and neck cancer treatments, and current research related to radiation-induced toxicities is mainly focused on patient experiences during radiation therapy. This study aims to analyze post-RT toxicity trends in working to identify social and clinical-based predictors of xerostomia and dysphagia development in HNC patient populations.

Forty-four head and neck cancer patients, who had undergone radiation therapy, were analyzed in this study. All study participants were over the age of eighteen and presented evidence of dysphagia and xerostomia at some point prior or during treatment. The patients were assessed for xerostomia and dysphagia at the following time points: pre-RT (baseline), mid-radiotherapy, end-radiotherapy, three months post-RT, six-months post-RT, and twelve-months post-RT. The patients' objective and subjective outcomes were scored at each time point to reveal predictors in toxicity development.

Trends in dysphagia and xerostomia revealed that men over the age of fifty (50) were more likely to develop post-RT toxicities than other gender- or age-based demographics. For patients, who were current or former smokers, the incidence rate was greatly amplified due to the potential for disease recurrences. The study also revealed that HPV positive patients were less likely to develop long-term dysphagia and xerostomia, while therapeutic combinations involving surgery with chemotherapy and/or radiation therapy presented greater concerns overall.

The presentation of this study will serve as a model for understanding the progression of radiation-induced toxicities—dysphagia and xerostomia—in HNC patients. Through identifying and addressing these predictors, future studies can be directed towards creating more target-based mechanisms for enhancing cancer survivorship and maintaining a patient's overall quality of life. With more funding, research support, and patient participation, a greater number of toxicity trends can be evaluated in working to create a more robust secondary analysis.

Keywords: Xerostomia, Dysphagia, Toxicity, RT, Development

CP RTP Summer Research Experience

Abstract Number: 19

**Analyzing Body Fat in Participants from Project TONE: An Exercise and Diet Intervention to Improve Body Composition in Postmenopausal Women with Normal BMI but Higher Body Fat**

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**Background:** Obesity as indicated by a high BMI is associated with an increased risk of breast cancer in postmenopausal women. However, BMI is not necessarily a precise indicator of breast cancer risk because it does not measure body composition. Iyengar et al. conducted an analysis of body fat levels in a subset of 3460 postmenopausal women with normal BMI ages 50-79 from the Women's Health Initiative (WHI) conducted 1993-1998. This analysis demonstrated that in postmenopausal women with a normal BMI, higher body fat is associated with an elevated risk of breast cancer. Higher body fat promotes breast cancer risk through both local changes—including inflamed adipose tissue and an altered microenvironment—and systemic changes—including circulating metabolic and inflammatory factors. Therefore, the goal of our ongoing study Project TONE is to determine whether a 16-week exercise and diet intervention can reduce body fat and thus cancer risk in postmenopausal women with normal BMI but higher body fat, a population under targeted in traditional interventions. For Project TONE, we are recruiting postmenopausal women ages 50-69 from the employee pool at MD Anderson. Interested women with a normal BMI will undergo a DXA scan to measure their body fat levels and determine whether they have a high enough body fat mass to participate (trunk fat mass  $\geq 9.4$  kg). I aimed to compare body fat distributions between postmenopausal women with normal BMI screened for Project TONE and those analyzed using data from the WHI.

**Methods:** For postmenopausal women with normal BMI screened for Project TONE, I calculated the quartiles of trunk fat mass, age, and BMI. I then compared these values to the same values in women analyzed using data from the WHI.

**Results:** I found that the first and second quartiles of trunk fat mass were higher in postmenopausal women with normal BMI screened for Project TONE than in women analyzed using data from the WHI. Additionally, the ages of women screened for Project TONE were lower and BMI values higher on average than women analyzed using data from the WHI.

**Conclusion:** Body fat levels are potentially higher now (2020s) than before (90s), which implies that lifestyle strategies are needed to promote favorable body composition phenotypes (e.g., lower body fat, adequate muscle mass). In the future, we hope to assess the efficacy and feasibility of Project TONE, as well as continue to analyze body fat variables in screened participants.

**Keywords:** postmenopausal women, intervention study, breast cancer, BMI, body fat

CP RTP Summer Research Experience

Abstract Number: 20

## **Using Novel Carbon Monoxide Devices in Remote Smoking Cessation Treatment: A Utilization and Reliability Analysis**

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**Background:** Smoking is the leading cause of preventable death in the world and accounts for a third of cancer-related deaths. Since 13% of the adult population are smokers, many researchers of smoking cessation have aimed at conducting smoking cessation research remotely, without the need of in-person visits. This method of remote intervention has much potential for reducing smoking prevalence, particularly by treating smokers who lack access to a smoking cessation clinic. For remote interventions, the standard measurement of verifying smoking abstinence is breath derived carbon monoxide (CO), a biochemical indicator of smoking/abstinence status. In conventional in-person smoking research, measuring CO requires special laboratory equipment, which is not accessible to average smokers. The recent development of portable devices has enabled smartphone-based CO equipment with an affordable cost. Researchers have been adopting smartphone-based CO measurement equipment for smoking cessation research. Though, little is known about the utilization and reliability of these novel mobile CO devices in smoking cessation research. This project will seek to discover if these smartphone-enabled CO devices are utilized and produce reliable measures among community smokers enrolled in a remote smoking cessation trial.

**Methods:** In a remote trial for smoking cessation, participants were recruited from across the entire state of Texas. Each participant was assigned with a smartphone-based CO device and instructed to use the CO device at specific time points during the treatment course. The obtained CO readings were uploaded automatically to our database hosted at MD Anderson Cancer Center. We conducted a secondary analysis to evaluate the utilization and reliability of smartphone-based CO measurements. We approached the procedure with participant assignment, data collection, and lastly the processing and data analysis utilizing Python-based tools.

**Results:** We examined the frequency of using the smartphone-based CO devices for utilization analysis and the correlation between CO levels and self-reported daily cigarette consumption for reliability analysis. We found that participants used the mobile CO devices more than required and CO levels were significantly correlated with the self-reported daily cigarette consumption. Additionally, a CO level of <6 ppm was the best discriminating cutoff to differentiate smoking from abstinent status.

**Conclusion:** Participants were able to utilize the smartphone enabled CO devices in a real-world trial more than required and showed interest in tracking their CO records. Smartphone-based CO devices provided reliable measures to identify smoking abstinence and serve as the future basis in remote smoking cessation intervention

**Keywords:** smartphone-based CO devices, Carbon Monoxide Devices, Novel CO devices, remotesmokingcessation

CP RTP Summer Research Experience



Abstract Number: 21

### **Risk Assessment Model for Breast Cancer in Women Using MERIT Cohort Study**

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**Background:** Breast cancer is the most common cancer among women and is the leading cause of cancer mortality. This stipulates screening mechanisms to promote early detection of breast cancer. The MERIT cohort study is intended to improve breast cancer detection for all women. It is based on women who are at risk and have been receiving annual screening. They are between ages 25-80, having not had breast cancer or breast feeding within the last six months. The study incorporates a questionnaire to the participants for additional data collection. Cases were identified during the study. This work applies the Gail Model [1,2] and a variant to identify risk factors using the MERIT cohort. The Breast Cancer Risk Assessment Tool (BCRAT) [3] developed by the NCI for the Gail Model is widely used to assess individual breast cancer average risk [4,5]. We develop a novel approach called the MERIT Cohort Risk Model (MCRM), a variant that includes additional risk factors and compares it against the Gail Model performance.

**Methods:** MERIT cohort study consists of 6298 women who have been participating from 2017. The age range is between 25-81 having not had breast cancer. These participants regularly take part in diagnostic screening. Additional risk factors gathered include breast density, BMI, menopause status, and race/ethnicity. 101 of the 6298 were reported as cases through early detection. Using SAS, the BCRAT tool was applied to build the Gail Model and understand relative risks. Subsequently, we build MCRM model using GLM to identify individual risks.

**Results:** The Gail Model showed significant risk factors. Using relative risks, the number of relatives  $\geq 2$  was significant (RR: (1.0006, 1.0031), 95% CI (1.0005, 1.00031). MCRM model showed better results with additional risk factors like breast density, BMI, and menstrual status. The interaction between breast density and age significance for women less than 50 (RR: 1.0052, 1.0066). No other measures showed significant risk after adjusting for loss to follow-up.

**Conclusion:** In conclusion, MCRM model provides better risk prediction by using additional risk factors in the MERIT cohort. MCRM is the first model that is based entirely on data specific to the MERIT cohort. Further studies with respect to race and number of second-degree relatives having breast cancer are warranted to evaluate the validity of interactions that may be established.

**Keywords:** MERIT cohort, Gail Model, Relative Risk, GLM

CP RTP Summer Research Experience

Abstract Number: 22

### **Evaluating patient engagement with a digital intervention for adolescent and young adult (AYA) stem cell transplant survivors**

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**Background:** Over time, advances in stem cell selection techniques have increased the viability of hematopoietic stem cell transplantation (HSCT) as a treatment option for leukemia and lymphomas including in adolescents and young adults (AYAs). AYAs face specific psychosocial and medical challenges in their HSCT treatment and prolonged recovery, which requires rigorous self-care and management following hospital discharge. These challenges in recovery can delay important milestones in education, employment, and relationships. Interventions to alleviate these barriers during their treatment have been introduced, yet evidence-based interventions focusing on promoting psychosocial and physical health after treatment and during recovery have yet to be established. Stempowerment is an online, interactive intervention developed to reduce barriers and increase motivation for self-care behaviors in AYA HSCT survivors, with the goals of optimizing medication adherence, hydration, physical activity, and psychological well-being post-discharge. As part of a mixed-methods process evaluation, we evaluated study enrollment plus engagement with and completion of the intervention in order to identify potential problems, barriers, and strategies for improvement.

**Methods:** To address current difficulties with patient recruitment and retention, process evaluation assessed study recruitment and refusal rates, participants' website access and social interactions, completion of intervention components (i.e., "Quests") and surveys, and end-of-study interview. Demographic and clinical data were obtained from medical and study records. A literature review was performed to evaluate published interventions for AYAs HSCT survivors and relative burden.

**Results:** Most participants recruited to the study completed the baseline survey and completed at least one quest, however, only 4 (17.4%) completed all quests. Additionally, most online social interactions were limited to sharing quest completions. Consistent with other studies, common reasons for refusal or withdrawal include illness severity and feeling overwhelmed. Univariate analyses comparing those who enrolled, refused, or withdrew revealed no differences in demographic or clinical variables.

**Conclusion:** To address these findings, our participant communication plan was revised to increase text messaging reminders and include varied messages reinforcing participation. Preliminary qualitative evidence suggests increased completion of individual quests and social interactions appear to be more frequent and may reflect greater engagement with the intervention and other users. Strategies to improve participation and engagement along with implementing frequent process evaluations with digital interventions are needed, particularly for patients coping with serious illness and difficult treatments.

**Keywords:** psychosocial, intervention, cancer, Hematopoietic stem cell transplant, AYA

CPRTP Summer Research Experience

Abstract Number: 23

### **The Effects of COVID-19 Restrictions and Community-Based Participatory Research, Delays in Project Self Recruitment**

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**Background:** The COVID-19 pandemic has had an overwhelming effect on Community-Based Participatory Research (CBPR), which ultimately relies on partnerships between community members, scientists and researchers to improve health. Project Self is a CBPR study with the overall goal to improve the cervical screening uptake rates among Hispanic and African American women living in Houston, Texas. This study proposed to complete all recruitment/enrollment and data collection efforts in-person at participating Houston Housing Association (HHA) housing sites (Fulton and Irvinton Village); which became a barrier with the onset of COVID-19 and the institutional and housing site restrictions.

**Methods:** This research study utilized literature reviews and in-depth interviews to learn about barriers faced by MD Anderson faculty/staff related to the COVID-19 pandemic and mandates put in place regarding community-based participatory research procedures.

**Results:** Findings provided during the in-depth interviews discussed challenges of community based-participatory research during the COVID-19 pandemic. Data collection strategies included literature reviews (n=5) and in-depths interviews with MD Anderson faculty/staff (n=2).

**Conclusion:** In an effort to make up for the time the study staff were unable to complete study procedures in the community, the main focus is to enhance Project Self community engagement strategies pertaining to recruitment/enrollment procedures at Fulton and Irvinton Village. We plan to stay engaged with the Houston Housing Authority (HHA) staff, Resident Council members, housing staff and residents by participating and contributing to upcoming community events and facilitating in-person meetings in the community centers to also assist with study implementation.

**Keywords:** Cervical Cancer, HPV, Community Based Participatory Research, Community Engagement  
CPRTP Summer Research Experience

### **PARPis As Immune Modulating Agents in Preventing Ovarian Cancer Recurrence**

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**Background:** According to the American Cancer Society, in 2022 about 21,000 women will receive an ovarian cancer diagnosis this year and about 13,000 women will die from ovarian cancer. And unfortunately, up to 85% of the women who undergo aggressive treatment – surgery, chemotherapy, and/or radiation – for advanced ovarian cancer face the risk of recurrence after initial treatment. However, Poly(ADP-ribose) polymerase (PARP) inhibitors (PARPis) are currently approved for the treatment of advanced breast cancers, ovarian cancers, and pancreatic tumors harboring BRCA1 or BRCA2 (BRCA1/2) mutations [1-6]. So, right now these drugs only help about 50% of patients with ovarian cancer; specifically, those who have completed initial treatment with surgery and chemotherapy and have homologous recombination deficiency (HRD) or BRCA mutations. So, it is important to find more biomarkers and targetable PARPs for patients with other forms of ovarian cancer to benefit from the cancer preventive effects of PARPis. Interestingly, recent published studies from Dr. Guang Peng's laboratory and other groups have revealed a previously unknown function of PARPis as immunomodulating agents through activation of the DNA sensing cyclic GMP-AMP Synthase (cGAS)-stimulator of interferon genes (STING) pathway [7-10]. The activation of this immune response in turn enhances T cell proliferation and trafficking to the tumor microenvironment [11,12]. Thus, PARPis can indirectly stimulate antitumor immunity by enhancing the recruitment of cytotoxic T-cells in both BRCA wildtype and mutant cancer cells [10]. Based on preliminary studies, we hypothesize that PARPis will induce a transcriptional program of immune responses through the cGAS-STING-TBK1-IRF3 pathway and active anti-tumor immunity in BRCA wildtype ovarian tumors as a preventive agent for recurrence.

**Methods:** (1) Ovarian Cancer cell cultures will be used as a model to test molecular effects. BRCA1 knockdown cells will be used as a control and compared to BRCA wild type cells. These cells will be treated with different dosages of PARPis. (2) We will use cell models to test whether PARPis may lead to an enhanced innate immune signaling in ovarian cancer cells by using RT-PCR to detect CCL5 and CXCL10. (3) We will use cell models to test whether PARPis may lead to an enhanced innate immune signaling in ovarian cancer cells by using Western blot to detect p-TBK1/TBK1 and p-IRF3/IRF3.

**Results/Conclusion:** Results from this project will provide novel mechanistic insights into the therapeutic efficacy of PARPis as immunomodulating agents. This concept of the underlying mechanism of PARPis will provide strong rationale to develop predictive biomarkers and preventive effects of PARPis for patients with other forms of ovarian cancer beyond BRCA deficiencies.

**Results/Conclusion:** Results from this project will provide novel mechanistic insights into the therapeutic efficacy of PARPis as immunomodulating agents. This concept of the underlying mechanism of PARPis will provide strong rationale to develop predictive biomarkers and preventive effects of PARPis for patients with other forms of ovarian cancer beyond BRCA deficiencies.

**Keywords:** PARP Inhibitors, Ovarian Cancer, Recurrence, Immune Response, Prevention

CP RTP Summer Research Experience

Abstract Number: 25

## **Racial and Ethnic Disparities in Breast Cancer Patients: A Literature Review of Time to Treatment (TTT)**

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**Background:** Advances in the diagnosis and treatment of breast cancer (BC) have resulted in a shift towards earlier detection and increased survival over the past several decades. Despite these advances, disparities in BC outcomes by race/ethnicity persist. Delays in TTT have emerged as factors that explain in part disparities between racial and ethnic groups. We performed a literature review to summarize research on racial and ethnic disparities in time to BC treatment to identify opportunities for intervention.

**Methods:** The literature search was conducted on June 23, 2022, and utilized five online databases: Medline, Embase, PubMed, Web of Science, and Scopus. The following criteria for inclusion of original research articles were used in the search: Focus on delays in BC TTT & disparities by race/ethnicity; Peer-reviewed & published within the last 15 years (2011-2022); Published in English & conducted in the United States. Of 120 articles found from the search, 37 articles met the criteria for review. Data extracted from these articles were compiled into a spreadsheet in order to reveal similarities and patterns across studies. Data extracted included: purpose, sample size, sex, age, race/ethnicity, the outcome of interest, findings, etc.

**Results:** Of the 37 studies, the majority (19) focused primarily on TTT disparities among White and Black women only. Most other studies examined outcomes across broadly defined racial and ethnic groups, most commonly Black, Hispanic, and Asian/Pacific Islander (API). Minimal research on disaggregated racial and ethnic groups was identified. Notably, only 4 studies compared outcomes within API and Hispanic subgroups. Across studies, findings suggest that people of color are more likely to experience delays in time to treatment than their White counterparts. Nearly all identified studies (30) showed that Black patients were more likely to experience longer time from diagnosis to BC treatment compared to other groups.

**Conclusion:** Studies in this literature review strongly show that Black BC patients consistently have longer TTT, which may contribute to their poorer BC outcomes and worse overall survival. Most articles reviewed used broadly defined racial and ethnic groups such as Asians and Hispanics, however, these are considered umbrella terms that consist of subgroups just as Asians include Chinese and Japanese. Future investigations should disaggregate these groups into subgroups to identify disparities. Undoubtedly, timely treatment is a human right, and receiving it should not be delayed. Implementing interventions to reduce TTT for Black women with BC should be a priority.

**Keywords:** Breast cancer, Time to treatment, Race/ethnicity, Disparities

CPRTP Summer Research Experience

**Colorectal Cancer Risk: What Does an Existing Tool Reveal About Modifiable Risk Factors in Young Patients?**

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**Background:** The National Cancer Institute's Colorectal Cancer Risk Assessment Tool was developed to estimate 5-year and lifetime colorectal cancer risk in individuals between the ages of 45 and 85. Despite a significant increase in colorectal cancer incidence among individuals aged 50 and younger, the existing tool does not feature validated cancer risk estimations based on age for this population. Risk prediction of CRC for young adults based on behavioral inputs using an existing tool has not been investigated.

**Methods:** 563 adult patients aged 18-90 at University of Texas, MD Anderson Cancer Center diagnosed with colorectal cancer completed a health behavior questionnaire at the time of recruitment. Each participant's 5-year and lifetime risks were calculated by entering questionnaire data into the CCRAT. Health behaviors and characteristics were compared by three age groups, 18-<45, 45-50, and >50. The prevalence of 5-year and lifetime elevated risk was compared among each group.

**Results:** The age groups significantly differed in the number of 5-year (<0.001) and lifetime (<.05) risk estimates that exceeded the population average risk for colorectal cancer. Significant differences were observed among age groups in cancer type (colon, rectal, or colon and rectal concurrently), body mass index, race/ethnicity, yearly time spent engaged in moderate and vigorous activity, aspirin use, having a first degree relative diagnosed with colorectal cancer, number of cigarettes per day (among smokers), and age at time of smoking cessation (among smokers). Of note, sex, weekly vegetable intake, and smoking were not significantly different among age groups.

**Conclusion:** Based on the tool's ability to predict high risk in this population of early- and late-onset colorectal cancer patients, the risk factors contributing to early-onset colorectal cancer likely differ from those of late-onset colorectal cancer. Additionally, differences between early-onset patients and late-onset patients in physical activity, body mass index, and having a first degree relative diagnosed with colorectal cancer demonstrate several clinical implications and require further investigation.

**Keywords:** colorectal cancer, young-onset colorectal cancer, risk factors, risk assessment, early-onset colorectal cancer

CP RTP Summer Research Experience

Abstract Number: 27

### **Early pancreatic cancer detection with hyperpolarized magnetic resonance**

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**Background:** Pancreatic cancer is among the most aggressive forms of cancer with a 5-year survival rate of 11%. There is an unmet need for diagnostic markers to detect premalignant/early stages of pancreatic cancer. Recent hyperpolarized magnetic resonance studies at the University of Texas MD Anderson Cancer Center have shown promise in early detection of pancreatic cancer in animal models. In these studies, it has been demonstrated that injected hyperpolarized pyruvate was converted to lactate at a heightened rate during the early stages of pancreatic cancer in mice. However, these studies were limited by the spontaneous nature of the mouse models, which led us to alter this study by using an inducible mouse model.

**Methods:** Hyperpolarization experiments were performed with 1-<sup>13</sup>C-labeled pyruvate containing 15 mM trityl radical (OX63) using a commercial DNP polarizer (HyperSense, Oxford Instruments, UK) at 3.35T magnetic field and a temperature of 1.4 Kelvin. <sup>13</sup>C-spectra were obtained using a Bruker BioSpec 7T imaging scanner, which utilized a dual tuned (<sup>1</sup>H) volume coil and (<sup>13</sup>C) surface coil (Doty Scientific, SC). Area under the curve values were collected for each metabolite, and lactate-to-pyruvate ratios were compared. Three different inducible mice models were used for these studies: P48CreERT2 (control), P48CreERT2:LSLKras (KCi), and P48CreERT2:LSLKras:LSLP53 (KPCi). Mice were imaged at three time points: preinduction, 10 weeks postinduction, and 20 weeks postinduction. A tamoxifen induction system was implemented to induce mutation and allow for better regulation of the cancer.

**Results:** There was a significant increase in the 20-week lactate-to-pyruvate found within the KPCi mice model (preinduction: 0.22; 10 weeks: 0.24; 20 weeks: 0.33). There was also a significant difference in lactate-to-pyruvate between KPCi mice (0.33) and the KCi (0.25) and control mice (0.26) at 20 weeks. No significant differences were found at the preinduction or 10-week timepoints in any of the three models.

**Conclusion:** Hyperpolarized lactate-to-pyruvate ratios in these mice models correlate with the aggressiveness of pancreatic cancer. Future directions include collecting data from one more timepoint (30 weeks). We also hope to understand the specificity of this technique to perform hyperpolarized imaging in a chronic pancreatitis GEM model, a known confounder of pancreatic cancer. Clinical translation of this technique could enable physicians to detect pancreatic cancer at premalignant stages, thus improving patient outcomes and survival. We are initiating a clinical trial for early detection of pancreatic cancer at the MD Anderson high-risk pancreatic cancer clinic by utilizing a recently installed clinical DNP polarizer

**Keywords:** Hyperpolarization, Pancreatic Cancer, Pyruvate, Lactate

CP RTP Summer Research Experience

### **Determination of Clinical and Environmental Risk Factors in Early-Onset Colorectal Cancer**

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**Background:** Colorectal cancer (CRC) is the third most common and deadliest cancer. With the advent of CRC screenings in 1996, overall colorectal cancer incidence and mortality rates have markedly declined. Late-onset CRC (LOCRC) cases have also continued to steadily decline due to recommended screening particularly in patients 50 years and older. However, early-onset CRC (EOCRC) – defined as CRC in adults younger than 50 – incidences have increased, with 71% of EOCRC cases being diagnosed at stage III or IV, according to the Colorectal Cancer Alliance. EOCRC differs from LOCRC across various genomic, clinical, and molecular characterizations, with EOCRC exhibiting later presentation stages and a greater likelihood for metastatic onset at presentation. The increasing prevalence and mortality rates of EOCRC along with the scarce existing research into EOCRC highlights the need for further research into EOCRC risk factors and clinical characterizations. Studying patients' general and specific external environment (exposome) along with internal environment factors contributing to heightened EOCRC incidence enables us to identify key risk factors. Findings from patient clinical and demographic data, exposures to carcinogens, lifestyle habits, and gene mutations can be used to better inform younger individuals on lifestyle changes or prevention strategies that could reduce the risk of EOCRC. In addition, identifying molecular markers and characteristics of EOCRC can assist in early clinical evaluation, reducing the chances of progression to advanced-stage CRC.

**Methods:** Analysis of clinical patient information contained in Epic charts will help uncover potential genetic and biological predispositions, lifestyle risk factors, demographic backgrounds, symptoms, and screening details that can better reveal links between risk factors and heightened susceptibility to early-onset colorectal cancer (EOCRC). This information will be drawn from EOCRC patients either currently or formerly enrolled in CRC clinical studies. Pairing clinical data with results from immune infiltrates, microbiomes, RNA seq data, and other molecular or biochemical assays could be key in further differentiating EOCRC from late-onset colorectal cancer (LOCRC) risk factors. CRC patients enrolled in the MD Anderson Assessment of Targeted Therapies Against Colorectal Cancer (ATTACC) study have completed comprehensive surveys assessing patient habits (smoking, alcohol consumption, diet) and exposures to various industrial and chemical carcinogens. Investigating these data and comparing between EOCRC and LOCRC environmental exposures in patients could uncover factors more strongly correlated with EOCRC cases.

**Results:** This research project will combine analysis of clinical patient information, biochemical and genomic assays, and environmental survey assessments to identify key risk factors for early-onset colorectal cancer (EOCRC). Data will be analyzed through Excel pivot tables and R to yield results that could help further characterize EOCRC and its profile. Results show significant difference ( $p < 0.5$ ) in gasoline exposures, with late-onset colorectal cancer patients (LOCRC) exhibiting higher years of gasoline exposure than compared to those with EOCRC. Smoking duration was also significant between LOCRC and EOCRC, with LOCRC patients smoking cigarettes for more years than compared to EOCRC patients' smoking habits.

**Conclusion:** Results may be subject to recall bias, as data were obtained through a retrospective patient survey and patient recall of exposure duration and frequency may not be accurate. Furthermore, LOCRC patients are expected to have a positive association with greater carcinogen exposure, as they tend to be



older and therefore have had more chances to be exposed to or use carcinogens. Our study suggests that exposome results alone are not sufficient to reveal differences between early and late-onset CRC. Further research needs to be conducted on other risk factors or clinical and molecular characterizations to uncover the main drivers of early-onset CRC.

Keywords: colorectal cancer, early onset, carcinogen, environment, clinical

CP RTP Summer Research Experience

## **Identification of cardiovascular disparities among Black and Hispanic survivors of adolescent and young adult (AYA) lymphoma**

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**Background:** Survival rates for adolescent and young adult (AYA) cancer patients have improved in recent decades. However, treatments such as anthracyclines and chest radiation therapy carry adverse cardiotoxic risks, predisposing this survivor population to early-onset cardiovascular dysfunction. To date, research on treatment-related cardiovascular dysfunction has primarily focused on survivors of pediatric and adult cancers, particularly of European ancestry. Importantly, Black and Hispanic individuals exhibit higher cardiovascular disease risk factors within the general population, potentially augmenting the risk for adverse cardiac outcomes after cancer treatment. Therefore, it is critical to determine the cardiovascular burden among diverse AYA cancer survivors. Here, we assess the prevalence of cardiovascular endpoints in a diverse cohort of AYA lymphoma survivors, addressing a crucial knowledge gap in cancer survivorship.

**Methods:** Medical records of Hispanic, non-Hispanic Black, and non-Hispanic White AYA lymphoma survivors (diagnosed between age 15-39,  $\geq 2$  years from diagnosis) were systematically abstracted. Cardiovascular outcomes were analyzed by self-reported race/ethnicity. To account for genetic diversity, stratification into groups based on proportions of continental genetic ancestry (European, African, Amerindian) using existing genotyping data and the ADMIXTURE analytical program was performed. The mean proportion of each ancestry group across the three self-identified race/ethnicity populations was used as a cut-point for preliminary stratification.

**Results:** The study population included 322 AYA survivors (12.8% Black, 18.6% Hispanic, 68.6% White) diagnosed with Hodgkin lymphoma (56.5%), non-Hodgkin lymphoma (41.6%), or other hematologic cancer (1.9%). Self-identified Black survivors exhibited higher prevalence of hypertension ( $P=0.005$ ), pericardial effusion ( $P=0.026$ ), cardiomyopathy ( $P=0.002$ ), and congestive heart failure ( $P=0.017$ ), while self-identified Hispanic survivors had higher prevalence of hyperlipidemia ( $P=0.025$ ). The average BMI change (mean $\pm$ SD) from initial cancer diagnosis to last follow-up decreased for Black survivors ( $-0.4\pm 4.5$  kg/m<sup>2</sup>) and increased in Hispanic ( $2.0\pm 4.8$  kg/m<sup>2</sup>) and White ( $2.3\pm 3.9$  kg/m<sup>2</sup>) survivors ( $P=0.036$ ). Individuals with  $<4\%$  European genetic ancestry (95.2% self-identified as non-White) showed higher prevalence of hypertension ( $P=0.026$ ), pericardial effusion ( $P=0.010$ ), cardiomyopathy ( $P=0.002$ ), and congestive heart failure ( $P=0.027$ ), compared to those with  $\geq 4\%$  European genetic ancestry (98.6% self-identified as White).

**Conclusion:** Despite improvements in AYA cancer survival, adverse effects on cardiovascular health remain prevalent after treatment. With research largely limited to pediatric and adult patients of European ancestry, assessment of cardiovascular outcomes in Black and Hispanic AYA survivors is critical to inform clinical follow-up procedures. This analysis helps to delineate the cardiovascular burden in a diverse AYA lymphoma cohort, with further variation based on genetic ancestry.

Keywords: adolescent and young adult cancer, anthracyclines, cardiovascular function, lymphoma, cancer survivorship

CP RTP Summer Research Experience

Abstract Number: 30

### **The Association Between Persistent Poverty and Melanoma Mortality in Texas: A Retrospective Study Using Texas Cancer Registry Data**

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**Background:** Patients in United States counties with persistent poverty (PP,  $\geq 20\%$  of residents in poverty since 1980) experience significantly higher cancer associated mortality than individuals living in non-PP counties. While markers of lower socioeconomic status have been correlated to higher melanoma mortality, the relationship of persistent poverty and melanoma mortality has not been explored.

**Methods:** We obtained data from 87,713 patients diagnosed with melanoma between 2000-2018 from the Texas Cancer Registry. We used 2021 US Economic Development Administration data to identify PP counties. Mixed effect models were used to assess incidence-based melanoma-specific mortality (IMM) rates by PP, age, sex and race/ethnicity.

**Results:** In 2021, 56 of 254 Texas counties met PP criteria. Of those diagnosed with melanoma, 5,431 patients in PP counties experienced higher IMM (17.14%) than those in non-PP counties (11.34%, adjusted odds ratio (AOR) = 1.10, 95% CI: 0.94-1.28). Compared to Non-Hispanic (NH) Whites, NH Blacks experienced the highest IMM at 25.67% (AOR = 2.97, 95% CI: 2.45-3.62), followed by Hispanics (19.71%, AOR = 1.98, 95% CI: 1.82-2.16). The disparity in IMM increased with age, with the 80 years + age group having the highest mortality AOR of 2.53 (95% CI: 2.16-2.96). When exploring sex, more men (13.43%) than women (9.09%) died from melanoma in PP counties (AOR = 1.48, 95% CI: 1.42-1.55).

**Conclusion:** Age, race/ethnicity, sex, and PP are associated with significant disparities in melanoma mortality outcomes within our patient cohort. To address these disparities and develop targeted melanoma secondary prevention interventions, we will continue to explore how place-based social determinants of health correlate with melanoma outcomes.

**Keywords:** Melanoma, Mortality, Texas, Persistent, Poverty

CP RTP Summer Research Experience

Abstract Number: 31

### **The Relationships of Childhood Trauma and Post Traumatic Stress Disorder to Smoking Outcomes in Cancer Patients**

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**Background:** Childhood trauma and post-traumatic stress disorder (PTSD) have been found to be related to higher levels of nicotine dependence and poorer tobacco cessation outcomes compared to smokers in the general population without these comorbid factors. To our knowledge, the relationship of these factors to nicotine dependence and cessation outcomes has not been evaluated in cancer patients. The aims of the current study are to assess the relationship of PTSD and childhood trauma to nicotine dependence and tobacco cessation outcomes in cancer patients participating in the MD Anderson Tobacco Treatment Program (TTP). We also hope to determine whether the effects of PTSD and trauma on abstinence will be mediated by negative affect.

**Methods:** We will use data which includes diagnoses of cancer patients seen for psychiatric care in the TTP. Within a sample of 687 patients, 57 have a diagnosis of PTSD and 183 have a history of childhood trauma. Generalized linear models will be used to evaluate relationships between PTSD and childhood trauma with nicotine dependence and abstinence. Analyses will adjust for psychiatric comorbidity as well as smoking and demographic characteristics. We used mediational analysis to identify the indirect effects of PTSD and Trauma that is attributed to changes in negative affect.

**Results:** Overall, the results confirm the hypotheses that PTSD and trauma adversely affect smoking outcomes. For abstinence, both PTSD and trauma were significant predictors, controlling for demographics and other psychiatric comorbidities. We found trauma to significantly predict nicotine dependence, for both negative and positive affect, whereas PTSD significantly predicted negative affect. Levels of negative affect accounted for a small but significant proportion of the effect of PTSD and trauma on abstinence.

**Conclusion:** The 2014 Surgeon General's Report on the Health Consequences of Smoking highlighted the negative impact of continued tobacco use in cancer patients, following diagnosis, on cancer treatment outcomes and survival. It is important to understand whether comorbid PTSD and the experience of childhood trauma presents additional barriers to tobacco cessation in this population. This additional knowledge may also aid in the development of targeted tobacco treatments that address these factors in cancer patients, which may improve tobacco cessation outcomes in cancer patients with these comorbidities.

**Keywords:** PTSD, Trauma, Affect, Smoking, Quitting

CP RTP Summer Research Experience

Abstract Number: 32

**A population-based study comparing comorbid conditions and mental distress among Hispanic Adolescent and Young Adult cancer survivors to propensity score matched controls**

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**Background:** The adolescent and young adult (AYA) age range is key for psychosocial growth and development; therefore, the addition of a cancer diagnosis may cause AYA cancer survivors to face higher levels of psychosocial distress. Furthermore, AYAs in minority populations, such as Hispanics, may face even higher psychosocial distress and poorer health-related quality of life (HRQoL). Research has indicated that risky health practices and chronic health conditions may lead to poorer mental health. Few studies assess the impact of a cancer diagnosis and risky health practices on Hispanic AYA's mental health and HRQoL. Therefore, the aim of this study is to investigate the health practices and mental distress among Hispanic AYA cancer survivors in comparison with Hispanic AYAs without a history of cancer in order to develop targeted psychosocial interventions.

**Methods:** The study was conducted using a cross-sectional, matched case-control design using population data from the National Health Interview Survey (NHIS). The Kessler nonspecific psychological distress scale (K6) was used to identify mental distress. The 6-item K6 scale asks respondents how frequently within the past 30 days they felt nervous, hopeless, restless, worthless, sad, and that everything was an effort. The total score can range between 6 to 30, with lower scores indicating worse mental health. Propensity score matching was utilized to match Hispanic AYA non-cancer controls to Hispanic AYA cancer survivors. Chi-squared test was utilized to examine the distributions of sociodemographic and health practices variables between the two groups, multiple logistic regression was used to examine differences in psychosocial distress between matched Hispanic AYAs with cancer and without cancer.

**Results:** Hispanic AYA cancer survivors and controls were matched on survey year, age at diagnosis, sex, education, income level, insurant status, and marital status. After matching, the final study sample included 295 Hispanic AYA cancer survivors and 295 controls. Sociodemographic and health practices, such as alcohol status, were comparable between two groups. Hispanic AYA cancer survivors reported more comorbid conditions than their healthy controls. After matching and adjusting for comorbid conditions, Hispanic AYA cancer survivors reported poorer psychosocial health than matched controls ( $b = -0.898$ ,  $t = -2.236$ ,  $p = 0.026$ ).

**Conclusion:** Overall, these results displayed a higher prevalence of comorbid conditions and worse mental health outcomes for Hispanic AYA cancer survivors compared to their matched healthy controls. These results provide a rationale for how Hispanic AYA cancer survivors may benefit from tailored surveillance and the creation of targeted intervention programs to improve their mental health outcomes.

**Keywords:** Hispanic, adolescent and young adult cancer, mental distress, kessler distress scale, psychosocial distress

CP RTP Summer Research Experience

Abstract Number: 33

### **NPRL2: A New Target In Breast Cancer Treatment**

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**Background:** It has been well established that genomic instability and mutation is a major hallmark of cancer development (Hanahan & Weinberg, 2011). However, due to deficiencies in mismatch repair, most breast tumors do not display high rates of mutational burdens compared to other cancers (Li & Chen, 2018; Marra et al., 2019). This suggests there are alternative pathways better for evaluating risk for breast tumor progression. Notably, studies from our team and others have revealed that cytosol DNA fragmentation activates DNA sensing pathways which in turn activate the innate immune response (Schrock et al., 2019; Shen et al., 2015). Our group decided to investigate molecular determinants of the c-GAS/STING pathways to see if defects in the S-DDR may reveal biomarkers responsible for the development of intermediate breast tumors. After running a genetic screen, we selected NPRL2 as a top candidate in regulating the S-DDR. NPRL2 is a primary component of the GATOR1 complex which has been linked to tumor suppression through its inhibitory interaction with mTORC1 (Bar-Peled et al., 2013). Although it has been established that loss of NPRL2/GATOR1 function leads to activation of mTOR signaling, the definitive role of NPRL2 in regulating the S-DDR warrants further research.

**Methods:** We utilized immunohistochemistry (IHC) to compare molecular differences in tissue microarray slides with breast lesions at various stages of breast cancer. Additionally, we used bioinformatic analysis of breast cancer samples in The Cancer Genome Atlas (TCGA) and generated knockdown of NPRL2 cell lines using lentivirus. Finally, we ran q-PCR to see if NPRL2<sup>-/-</sup> or defects in the S-DDR promote innate immune signaling in breast cancer cells.

**Results:** Through our research, we found a positive correlation between NPRL2 and CHK1 expression in Triple Negative Breast Cancer (TNBC). Additionally, we discovered that reduced NPRL2 expression in breast cancer induced innate immunity and also correlated with a worse prognosis in breast cancer patients.

**Conclusion:** From these results, we identified NPRL2 as a mediator of the innate immune response and a contributor to cancer development. It is important to note that low levels of NPRL2 are associated with higher risk of breast cancer. Consequently, it is important that we identify these high-risk patients early so that treatment can be started before the cancer becomes advanced. Although more research needs to be done to evaluate NPRL2 as a possible target in pharmacological therapies for breast cancer, the association between the gene and tumor progression has been investigated through these experiments.

**Keywords:** NPRL2, breast cancer, innate immunity, DDR, CHK1

CP RTP Summer Research Experience

### **Evaluating Donor PBMC's expression of HLA-A, B, and Cs**

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**Background:** Cervical cancer is the fourth most common cancer seen in women. Nearly all cervical cancers are attributed to the Human Papillomavirus (HPV). HPV is divided into two groups, high-risk types, and low-risk types. HPV-16 is a high-risk type of the virus because it can result in cancer. Currently, cellular therapy is being developed and researched to improve the immune system's ability to fight off cancerous tumors. Once malignancies caused by HPV reach the metastatic stage they become incurable and resistant to standard therapies, such as chemotherapy and radiation. This occurs because the malignancies uniformly express the HPV E7 antigen, which contributes to cancer cell survival. Epitopes, also known as antigenic determinants, are protein domains that can elicit a cellular immunological response from T or B cells. T-cell epitopes are peptides produced from protein antigens that are presented by MHC molecules on antigen-presenting cells and recognized by T-cell receptors, which then elicit an immune response to invading pathogens. The Human Leukocyte Antigen (HLA) plays a huge role in the immune response to viruses/tumor cells by presenting self and non-self epitopes to the immune system. This may lead to the killing of the cell presented to the epitope on the HLA. T cells are unable to recognize tumor cells that do not express HLA, which allows the cells to escape the immune response through downregulation, resulting in malignancies. I will test a sample of Human peripheral blood mononuclear cells (PBMCs) HLA expression to see if I can then follow the same steps to test the HLA expression of tumor cells

**Methods:** PBMCs were obtained from a donor infected with HPV16 and battling cancer as a result. Cells were then stored in the nitrogen box until ready for use. Cells were collected from the nitrogen box and soaked in a warm 35-celsius bath until a small ice chunk was present. 10 ML of the prewarmed medium was added to the cells. The cells were then placed in the centrifuge and spun at 350 rcf, for 5 minutes, at room temperature. Using the drop-by-drop method when adding the medium to the cells will prevent osmotic shock. Next, the cells were placed into a flask and incubated in order for the cells to attach to the flask. Passaging the cells came next after the cells were successfully attached to the flask. The cells were then rinsed and a dissociation reagent (TRYPLE) was added. The cells appeared round when released from the substrate. Cells were then placed in the centrifuge and a newly formed pallet formed. The pallet was then resuspended with a growth medium. A small amount of medium was removed for a cell count. The cell count was performed by using Trypan blue, which gave the dead to alive cell ratio. I then stained the cells and used flow cytometry to measure the expression of HLA within my cells. I measured surface expression of HLA molecules using fluorochrome conjugated antibodies..Flow cytometry told me the intensity of HLA expression, rather than just yes/no if an expression is present. As the flow cytometry results were converted into a graph, a variety of T cells were tagged with different color probes so that they could be separately identified.

**Results:** The cells went through the staining process and were successfully run through the flow cytometry machine. The cells were stained for the expression of HLA-A, B, and C. Staining tested for the positive and negative controls of HLA expression within my cells. I proposed that not all of the cells would express HLA and that the levels of different HLA molecules are different. I said this because cells are very sensitive, when growing the cells they are under a great amount of stress and this could alter whether or not they will express HLA. After staining my cells and running them through Flow Cytometry I found that all of my cells from donor 1 expressed HLA A, B, AND C. I compared them against Isotypes A, B, and C to see if the expression of HLA was present.



Conclusion: I used healthy donor PBMCs to undergo the process of thawing cells, staining, and running the cells through flow cytometry. This experiment was important because it allowed me to understand the process that I later use with tumor cells. Flow cytometry allowed me to see the intensity of HLA expression within my cells, which is very important because HLA acts as the immune response to pathogens. When HLA is decreased/down-regulated mutations occur and pathogens are able to evade being presented to epitopes by HLA, which allows them to not be killed and form tumors.

Keywords: PBMCs, HLA expression, epitopes, cellular therapy

CP RTP Summer Research Experience

### **Elucidating the Role of Microbiome in Low- and High-Grade Glioma**

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**Background:** Glioma is a type of CNS tumor, originating from glial cells and further classified into grades I-IV. Low-grade gliomas are often characterized by mutant IDH while high-grade gliomas tend to express the wild-type. Glioblastoma (grade IV) is the most prevalent malignant adult brain tumor, characterized with a poor prognosis despite maximal therapy. While immunotherapy, specifically immune checkpoint blockade, has been effective in other solid malignancies, it has demonstrated limited success against glioblastoma, likely due to its immunosuppressive tumor microenvironment and intrinsic immune evasion. The gut microbiome has been demonstrated to be involved in the immune system and tumor immunity in various tumors. However, the role of the microbiome and microbiota-driven immune modulation in glioma is still largely unknown, presenting the opportunity to investigate the glioma development through the modulation of systemic and local immune responses.

**Methods:** Gut microbiome samples collected from 30 patients with low- and high-grade glioma at the time of surgical resection and sequenced via metagenomic shotgun sequencing were analyzed in order to determine the association of microbial signatures with tumor grade and tumor progression metrics. To determine the mechanistic role of the gut microbiome in shaping the immune microenvironment in the brain, we used a mouse model of gut microbiome depletion. This model consisted of mice treated with a cocktail of non-absorbable broad-spectrum antibiotics to deplete the gut microbiome and control mice treated with vehicle alone. Three weeks after the initiation of treatment, the animals were euthanized, brain tissue was analyzed for the local and systemic immune profiles using flow cytometry, and gut microbiome profiles were analyzed using sequencing.

**Results:** Clinical studies demonstrated patients with high-grade glioma and IDH-wild-type glioma had higher bacterial taxa diversity within the gut microbiota than low-grade glioma and IDH-mutant glioma, respectively, with enrichment of distinct taxa in low- vs high-grade and mutant vs wild-type IDH glioma. The pre-clinical studies demonstrated a decrease in gut microbiome diversity with antibiotic treatment administration. Evaluation of the local and systemic immune profiles suggested gut microbiome depletion in non-tumor-bearing mice induced an immunosuppressive environment in the brain.

**Conclusion:** Enrichment of distinct microbial communities is associated with glioma grade and IDH type. The absence of gut microbiota can modulate T cell and microglia activity regulation, inducing an immunosuppressive microenvironment in the brain. Future studies will investigate the distribution of cells and microbes in the tumor microenvironment using spatial transcriptomics to further elucidate the role of these microbial signatures in glioma growth.

**Keywords:** Glioma, glioblastoma, microbiome, immunotherapy

CP RTP Summer Research Experience

Abstract Number: 36

### **Lipocalin 2 protects from lung tumorigenesis associated with gut microbiota alterations**

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**Background:** Lung adenocarcinoma (LUAD), the most common cancer diagnosed in smokers, frequently exhibits somatic mutations in the *KRAS* oncogene. *KRAS*-mutant LUAD (KM-LUAD) displays dismal prognosis, warranting better understanding of its pathogenesis and new strategies for early prevention and/or interception. Growing evidence shows that the gut microbiome is a key player in modulating host immune response, tumorigenesis, and response to therapy. Yet, our knowledge of the gut-lung axis is still in its infancy. Our lab previously showed genetic deletion of *Gprc5a* leads to lung tumors harboring driver *Kras*<sup>G12D</sup> mutations, the same variant found in human KM-LUAD, and that are accelerated by exposure to tobacco carcinogens such as nicotine-specific nitrosamine ketone (NNK). Tumor burden after NNK exposure was associated with progressive and distinct changes in the gut microbiome taxonomical composition. Notably, we found that loss of the antimicrobial immunomodulatory *Lcn2* in NNK-exposed *Gprc5a*<sup>-/-</sup> mice (*Gprc5a*<sup>-/-</sup>/*Lcn2*<sup>-/-</sup>) led to increased tumor development, inflammation, as well as distinct microbial profiles with markedly reduced bacterial diversity. This suggested that LCN2 perhaps counteracts microbial dysbiosis and ensuing LUAD development.

**Methods:** We thus compared and contrasted tumor development in syngeneic lung cancer mouse models with that received fecal microbiota transfer (FMT) from *Gprc5a*<sup>-/-</sup>/*Lcn2*<sup>-/-</sup> (GL) and *Gprc5a*<sup>-/-</sup> (G) mice.

**Results:** We found that FMT from GL animals led to markedly increased growth of transplanted LUADs relative to animals that received feces from G animals with intact LCN2. This observation was consistent across different recipient syngeneic animals, both in *Gprc5a*<sup>-/-</sup> and 129-Elite wild-type mice. Increased tumor growth in animals receiving GL FMT was associated with pronounced mesenteric lymph nodes and presence of erythematous Peyer's patches suggestive of perhaps systemic immune and inflammatory cues linking gut microbial dysbiosis to LUAD growth.

**Conclusion:** Specifically, we show that LCN2 plays critical roles in maintaining gut microbiome homeostasis against bacteria that perpetuate LUAD development. Our study highlights functional roles for the gut microbiome in LUAD development. Our preliminary findings warrant further efforts to identify specific tumor-promoting bacteria that can potentially be targeted for the prevention and intervention of LUAD.

**Keywords:** lung adenocarcinoma, microbiome, lipocalin 2, smoking, carcinogenesis

CP RTP Summer Research Experience

Abstract Number: 37

### **Mindfulness-base Intervention for Latino Patients and their Caregivers**

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**Background:** Latinos with cancer are more likely to suffer from psychological morbidities compared to non-Latino white patients. Similarly, Latino caregivers also suffer from increased distress. However, Latino cancer patients report less use of psychosocial interventions compared to their non-Latino white patients. Latinos are severely underrepresented in psychosocial intervention research, resulting in limited knowledge on the feasibility, acceptability, and efficacy of mindfulness-based interventions in this population.

**Methods:** As part of a larger study to adapt a mindfulness-based intervention for Latino cancer patients and their family caregivers, this project evaluated aspects of feasibility of conducting a mindfulness intervention and explored strategies for coping with cancer in this population. Latino patient-caregiver dyads were recruited from MD Anderson's Oncology Program at Lyndon B. Johnson Hospital (LBJ). Where LBJ serves low-income and medically underserved patients. During Phase 1, rigorous formative research was conducted with 20 different Latino cancer patient-caregiver dyads. The dyads participated in a brief survey over the phone and a 1.5-hour formative research session that included a semi structured interview to explore cancer related experiences and two brief mindfulness exercises. ATLAS.ti was used to identify preliminary themes related to how patients and caregivers cope with cancer.

**Results:** The two main goal for this project include evaluating the feasibility of a mindfulness intervention for Latinos and to better understand how this population copes with cancer. Participants were primarily of Mexican origin (70%) and with low levels of education (30% reporting 8th grade or less). Although men and women were represented similarly among cancer patients, caregivers were predominantly women (60%). According to ATLAS.ti, religion and the support of family/friends were the two predominantly preliminary themes seen to cope with cancer between the dyads. Where some have even said that "The faith we have has helped us; we're a catholic family. I think that mutual support and faith in God, I think that's what has helped us stand on our feet."

**Conclusion:** The data collected suggests that mindfulness-based practice may be a feasible approach to decrease stress in Latino patients/caregivers, as well as give a understand on the effect of mindfulness-based practice in the Latino community. Findings will then be used in a later state to adapt to a larger study of Latino patient caregiver dyads in Phase 2.n:

**Keywords:** Psychosocial, Mindfulness, Latino, Morbidities, Mindfulness-Based Practice

CP RTP Summer Research Experience

### **Analysis of Primary and Secondary Ewing Sarcoma Outcomes**

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**Background:** Ewing Sarcoma (EWS) is a rare cancer of bone and soft tissue that predominately occurs in adolescents and young adults. EWS is often associated with a second malignancy, which can occur before or after the EWS diagnosis. EWS occurring after a prior malignancy is less common and understudied. We aimed to determine whether clinical presentations and long-term survival were different between primary and secondary EWS.

**Methods:** Patients diagnosed with EWS and confirmed by the EWSR-FLI1 fusion transcript by pathology reports at MD Anderson were analyzed by a retrospective chart review. Patients with a Peripheral Neuro-Ectodermal Tumor (PNET) diagnosis were not included in our cohort. Overall survival was determined by Kaplan-Meier methods and calculated using the log-rank test. Differences in average survival were calculated with two-sample t tests.

**Results:** Twenty-two cases of secondary EWS were reported, accounting for 47.8% of all EWS cases. Patients with secondary EWS were diagnosed with EWS at an older age than patients with primary EWS (47.9 years vs 30.6 years,  $p = 0.004$ ). Patients with secondary EWS had a significantly lower five-year survival rate (16.7% vs 62.2%,  $p < 0.001$ ). However, patients with secondary EWS had a longer time gap between malignancies than patients with primary EWS, (109.7 months vs 39.5 months,  $p = 0.001$ ). Secondary EWS patients had a significantly worse response to radiation therapy than primary EWS patients (22.6 months vs 113.5 months,  $p = 0.001$ ). Average survival was significantly longer in secondary EWS patients without metastasis compared to secondary EWS patients with metastasis (36.4 months vs 15.4 months,  $p = 0.023$ ).

**Conclusion:** Secondary EWS patients are diagnosed with EWS at an older age, have poorer long-term survival, take more time to develop a future malignancy, and respond worse to radiation therapy than primary EWS patients. These findings indicate that secondary EWS patients are distinct from primary EWS patients and possibly have a unique germline mutation predisposing them to EWS.

**Keywords:** Ewing, Sarcoma, malignancies, survival

CP RTP Summer Research Experience

**Psychosocial mediators of the association between distress related to COVID-19 and physical activity among rural cancer survivors**

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**Background:** The COVID-19 pandemic notably impacted cancer survivors and those living in rural areas more severely than those without a history of cancer and those living in urban settings. Cancer survivors and rural adults reported lower quality of life and higher psychosocial distress during the pandemic. Furthermore, higher distress is associated with physical inactivity, which can further worsen overall health. Previous studies have looked at the association between COVID-19 distress and physical activity. However, few have examined this association in rural cancer survivors. This study explored associations between COVID-19 distress and physical activity among rural cancer survivors and explored psychosocial mediators of this association. We hypothesized that higher distress related to COVID-19 and poorer psychosocial wellbeing were associated with lower physical activity in rural cancer survivors and that psychosocial wellbeing partially mediated the association between COVID-19 related distress and physical activity.

**Methods:** This was a secondary data analysis from the Partnering to Prevent and Control Cancer (PPCC) study. Rural cancer survivors (n=219) residing in central Pennsylvania completed a mailed questionnaire to assess health behaviors and ecologic determinants in 2017-2018. Participants who provided a working email address (n=195) were re-contacted in April 2020 and completed an online questionnaire assessing changes in health behaviors, psychosocial wellbeing and psychosocial distress related to COVID-19. Those who completed this questionnaire (n=104) were included in this study. Linear regression models were used to assess associations between COVID-19 distress and physical activity, and psychosocial wellbeing and physical activity, controlling for age, gender, BMI and education. Mediation analyses included four single-mediator models to assess the indirect effects of COVID-19 distress on physical activity through psychosocial wellbeing (anxiety, depressive symptoms, perceived stress, and negative affect).

**Results:** Participants were an average age of  $61.9 \pm 13.9$  years, mostly women (65.4%), and 63.4% having a bachelors or higher education. Most participants were breast (26%) or prostate (20.2%) cancer survivors. After controlling for covariates, greater COVID-19 distress was associated with higher (walking) physical activity ( $B=80.8$ ,  $SE=39.4$ ,  $p=.043$ ). Anxiety ( $B=-38.9$ ,  $SE=17.6$ ,  $p=.030$ ), depressive symptoms ( $B=-33.0$ ,  $SE=14.7$ ,  $p=.028$ ), and negative affect ( $B=-139.6$ ,  $SE=67.9$ ,  $p=.043$ ) were all negatively associated with (moderate/vigorous) physical activity. Single-mediator models showed anxiety (Indirect Effect=-71.6,  $SE=20.7$ , 95% CI=-122.8, -30.5), depressive symptoms (Indirect Effect=-46.6,  $SE=16.4$ , 95% CI= -79.2, -14.0), and negative affect (Indirect Effect=-187.9,  $SE=77.3$ , 95% CI=-341.5, -34.3) significantly mediated associations between COVID-19 distress and physical activity.

**Conclusion:** Rural cancer survivors who reported greater COVID-19 related distress reported higher anxiety and depressive symptoms, which was associated with lower levels of physical activity. Findings point to the need for psychosocial interventions that address pandemic-related distress to improve physical activity in rural cancer survivors. Additional studies are needed to better understand how to improve psychosocial wellbeing to reduce distress and increase physical activity to reduce cancer health disparities in rural communities.

**Keywords:** Psychosocial Distress, Physical Activity, COVID-19, Mediation

CP RTP Summer Research Experience

Abstract Number: 40

### **Multiple levels of influence on Asian American colorectal cancer screening**

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**Background:** Although the United States has seen its Asian population nearly double to approximately 19 million over the past 20 years, Asians remain severely underrepresented in colorectal cancer screening when compared to other racial groups. This is especially concerning given Asians are the only United States racial group where cancer is the leading cause of death in both males and females. To effectively improve colorectal cancer screening among Asian Americans, it is important to understand factors, beyond knowledge, associated with their colorectal cancer screening across multiple levels of influence. Thus, the aim of this study is to review what factors across individual, physician, cultural, and healthcare levels are associated with colorectal cancer screening.

**Methods:** The electronic database PubMed was searched. Eligible studies were coded across 4 levels of influence: (a) individual demographic psychological, such as perceived risk of colorectal cancer and embarrassment (b) physician (e.g., physician recommendation, physician trustworthiness), (c) cultural (e.g., acculturation), and (d) healthcare (e.g., insurance status, having a primary care physician).

**Results:** Of the initial 47 studies identified in the PubMed search, 13 studies, all of which were cross-sectional, were deemed eligible for review. Most eligible studies collected data on a single race/ethnicity (n=9/13), while the remaining studies (n=4/13) included multiple racial/ethnic groups. Both papers analyzing perceived colorectal cancer risk showed a positive association with colorectal cancer screening, while both papers analyzing knowledge score found no significant association. Physician recommendation was shown to have a positive association with colorectal cancer screening in all five papers analyzing the impact of recommendation, while having routine check-ups was also shown to have a positive association in all three papers analyzing check-ups. Acculturation, which encompasses English fluency and duration in the United States, was shown to have a positive association with colorectal cancer screening.

**Conclusion:** All four levels of influence (Individual demographic psychological, physician, cultural, and healthcare) were shown to be associated with Asian American colorectal cancer screening. However, because the acculturation paradox suggests increased duration in the United States is associated with decreased health, contrary to the positive association found with colorectal cancer screening, more research is needed to show how acculturation impacts Asian American cancer related health.

**Keywords:** Asian American, colorectal cancer, screening rates, multiple levels of influence, health disparities

CP RTP Summer Research Experience

Abstract Number: 41

### **Personalized Lung Cancer Screening - Acceptability among Primary Care Providers**

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**Background:** Lung cancer screening via LDCT (low dose computed tomography) has been shown to significantly decrease lung cancer-specific mortality. Current LCS (lung cancer screening) guidelines are reliant solely on a patient's age and smoking history. Despite its proven benefit, only 5-15% of eligible patients receive LCS, with Texas having one of the lowest rates in the United States. Recent advancements in screening methodologies suggest that personalized approaches can increase screening's effectiveness, reduce false positives, and reduce the net screening examinations required per life-year obtained. This study assesses the current practice of and acceptability of a personalized approach for LCS among primary care providers in Texas.

**Methods:** We developed and distributed a survey to 32983 Texas-based primary care providers on an existing network (Protocol 2019-1257; PI: Dr. Sanjay Shete) including primary physicians, physician assistants, and nurse practitioners. Deidentified demographics, information regarding current LCS practices, and acceptability of a personalized approach were obtained. Responses were broken down and analyzed in sub-categories using comparative statistics and a 10x bootstrapped logistic regression to further understand respondent tendencies by subgroup. Thirty willing respondents will be randomly selected and interviewed to investigate survey responses, opinions of LCS, and feedback on a decision tool that delivers personalized screening decisions.

**Results:** There were 91 (0.3%) respondents. The demographic data included: female 74%. 72% White, 14% Black, 12% other, with 11% being Hispanic. There were relatively even numbers of physicians (32%), PAs (23%), and NPs (44%). We found that 70% of respondents provided LCS services into their practice and 48% were interested in adopting a personalized approach to LCS screening, with 40% unsure about the personalized approach. Majority (62%) of respondents expressed concern about the increased time of the personalized approach. Comparison of each age group among providers (40-49, 50-59, 60-69) to providers under 40 using the logistic regression found positive correlation between provider young age and interest in personalized LCS ( $p < 0.05$ . OR 0.259, 0.594, and 0.081 respectively).

**Conclusion:** Of the primary care providers who responded, the majority included some elements of screening into their practice. Nearly half of the respondents were open to adopt a personalized approach to LCS screening. Time limitation was the main concern, and if addressed, acceptability of personalized LCS may potentially reach 70-90%.

**Keywords:** Lung cancer screening, risk based approach, acceptability, current practice

CP RTP Summer Research Experience



Abstract Number: 42

### **Barriers with Implementing the Federal Smoke-Free Public Housing Rule at Cuney Homes, Houston's Largest Public Housing Site**

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**Background:** Second-hand smoke exposure (SHSe) in non-smokers increases the chances of developing lung cancer by 20-30%. Since even brief smoke exposure is carcinogenic, there is no risk-free level of SHSe. To address the higher rates of SHSe in public housing communities, the U.S. Department of Housing and Urban Development (HUD) implemented the Smoke-Free Public Housing Rule (HUD-SFPHR) in 2018, which mandated that all public housing sites prohibit smoking inside residential and communal buildings, as well as within 25 feet of these properties. Previous research indicates that HUD-SFPHR has not been entirely effective, so this study aims to identify barriers interfering with the implementation of HUD-SFPHR at Cuney Homes, Houston's largest public housing site with an 89.5% African American/Black residential population.

**Methods:** Property observations at Cuney Homes were performed and the number of cigarette litter, HUD-SFPHR signage, and active smokers were recorded. Furthermore, in-depth staff interviews (n=2) were conducted to learn about the implementation of HUD-SFPHR at Cuney Homes.

**Results:** During the property observations, 2,311 pieces of cigarette litter, 15 active smokers, and 0 HUD-SFPHR signage were found. The in-depth interviews revealed that residents are not aware of HUD-SFPHR due to a lack of education provided about the rule, staff are hesitant to have conversations with residents about tobacco use, and there are currently no tobacco treatment services or resources available to residents. Furthermore, due to the COVID-19 pandemic there have been no property health inspections since 2019, which has led to a lack of violations being given out for breaking HUD-SFPHR. Many residents also fear retaliation from their neighbors, so they do not complain about SHSe.

**Conclusion:** The property observations revealed that residents at Cuney Homes are not adhering to HUD-SFPHR. Based on the barriers identified from the interviews, staff need to be trained to educate residents about HUD-SFPHR, SHSe, & the risks associated with tobacco use during New Resident Orientation. There also needs to be a bigger emphasis on encouraging tobacco treatment. This can be done by promoting community-based cessation programs and offering culturally tailored resources which talk about menthol cigarette use and big tobacco marketing, both of which have disproportionately affected African American/Black communities. Through Project Smoke-Free, we will be evaluating how implementing these recommendations will improve resident adherence of HUD-SFPHR. Overall, addressing the barriers in implementing HUD-SFPHR may reduce the disproportionate amount of lung cancer and other tobacco-related health conditions in African American/Black and low-income/underserved communities.

**Keywords:** smoke free public housing rule, public housing, intervention, lung cancer, African American  
CP RTP Summer Research Experience

Abstract Number: 43

## **Understanding the Impact of COVID-19 on Psychosocial Distress and Quality of Life Among Rural Cancer Survivors**

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**Background:** COVID-19 has impacted millions of people globally, and cancer survivors and underserved populations face increased risk of infection and death. Rural adults and cancer survivors, who are at greater risk of COVID-19, experience greater psychosocial distress. However, COVID-19's impact on psychosocial distress and quality of life (QOL) among rural cancer survivors has gone unexplored. This study examined (1) changes in psychosocial distress and QOL in rural cancer survivors during COVID-19 and (2) associations between changes in psychosocial distress and QOL and health behaviors among rural cancer survivors in central Pennsylvania.

**Methods:** This study is a secondary data analysis from the Partnering to Prevent and Control Cancer (PPCC) study (2017-2018) and the follow-up COVID-19 Impact Study (April-May 2020). Rural cancer survivors (n=219) in PPCC completed questionnaires assessing individual, social, and environmental factors that affect health behaviors. A subsample of PPCC participants (n=104) completed the follow-up questionnaire, which assessed the impact of COVID-19 on health behaviors and psychosocial distress. Changes in psychosocial distress and QOL from pre- to post-pandemic were evaluated using paired samples t-tests. Multiple regression models were used to explore the association between changes in psychosocial distress and QOL and health behaviors (physical activity, sleep quality, fruit and vegetable intake, and alcohol use) during the pandemic, adjusting for age, gender, BMI, and education.

**Results:** Rural cancer survivors were in their early 60s (M=61.86±13.9) and mostly women (65.4%). The majority were breast (26%) or prostate (20%) cancer survivors, not currently receiving cancer treatment (92.2%), and at least 3 months post treatment (95.1%). Participants reported decreased perceived stress ( $\Delta=0.3$ ) and positive affect ( $\Delta=0.4$ ) but increased depressive symptoms ( $\Delta=1.4$ ), anxiety ( $\Delta=0.5$ ), negative affect ( $\Delta=0.3$ ), and general health ( $\Delta=0.8$ ). However, changes were not statistically significant ( $p>0.05$ ). Changes in perceived stress ( $b=-0.180$ ,  $p=.004$ ) and negative affect ( $b=-0.139$ ,  $p=.030$ ) were negatively associated with post-COVID alcohol use, and anxiety ( $b=0.164$ ,  $p=0.047$ ) was positively associated with post-COVID sleep quality.

**Conclusion:** Rural cancer survivors did not report statistically significant changes in psychosocial distress or QOL, and the minimal changes reported were associated with healthful behaviors, including reduced alcohol use and better sleep quality. Cancer survivors residing in rural areas may have experienced less drastic lifestyle changes due to lockdowns early during the pandemic, contributing to healthful behaviors. Additional research is needed to explore the impact of COVID-19 on psychosocial wellbeing and health behaviors further into the pandemic to determine the long-term effects.

**Keywords:** Rural Health Disparities, Cancer Prevention, Psychosocial Distress, Quality of Life, Health Behaviors

CP RTP Summer Research Experience

Abstract Number: 44

**Skin cancer surveillance patterns of actinic keratoses patients in US Medicare population: a cohort study**

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**Background:** Actinic Keratoses are precancerous skin lesions that are very common. Patients with actinic keratoses (AKs) are at higher risk for developing skin cancer. However, the follow up patterns and skin cancer surveillance for AK patients are relatively unknown in the US.

**Methods:** This retrospective cohort study used de-identified United States Medicare claims data from 4,999,999 beneficiaries aged  $\geq 65$  from 2009 to 2018. Participants had to have been in the dataset for one year prior to an AK diagnosis and had to be in the dataset for at least one year after that AK diagnosis. Participants were identified using outpatient AK diagnosis codes. Number and percentage of patients who had total body skin checks/skin cancer screenings identified by: outpatient dermatology visits, visits with skin cancer screening diagnosis codes, or visit with one or more of the commonly used dermatology codes, within 12 months after principal AK diagnosis.

**Results:** 64.14 % of patients with at least one actinic keratoses diagnosis were seen back for a skin cancer surveillance exam within 12 months.

**Conclusion:** AK follow up skin exam frequencies are lower than what we expected for 12 months. Understanding the current patterns in skin cancer surveillance for AK patients is important to guide the creation of evidence-based recommendations for this high-risk population.

**Keywords:** Skin cancer prevention, Actinic Keratoses,

CP RTP Summer Research Experience

**Association Between Rare TP53 variants and Cancer Predisposition in Colorecta, Melanoma, Ovarian, Pancreatic, and Prostate Cancer**

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**Background:** Pathogenic variants in the TP53 gene lead to Li-Fraumeni syndrome (LFS). TP53 codes for tumor suppressor proteins that control cell division, DNA repair, and apoptosis. When variants of this gene occur, the proteins can be prevented from performing their function. Individuals with LFS have a very high risk of developing multiple cancers, including breast cancer, bone cancer, leukemia, lung, and sarcomas. However, TP53 carriers can be at risk for developing other cancers which are not typically linked with this syndrome. This study took a closer look at rare TP53 variants through a sequencing case-control studies in colorectal, melanoma, ovarian, pancreatic, and prostate cancer to evaluate potential association between variants and cancer predisposition.

**Methods:** DNA from a total of 13,396 cases and up to 7,788 controls from the University of Texas MD Anderson Cancer Center (MDA), H. Lee Moffitt Cancer Center & Research Institute, The University of Utah School of Medicine, Duke University, and UK Biobank were sequenced. All controls had no prior history of cancer and cases reported that the tumor they were categorized under was their primary tumor. We conducted gene-based p-values to evaluate associations with rare, protein-coding TP53 variation and each cancer using VAAST. We also calculated effect size estimates for three categories of variants in each cancer as classified by ClinVar: pathogenic (including novel truncating variants), variants of uncertain significance (VUS), and benign. For pathogenic variants, we also derived more precise risk estimates through a meta-analysis incorporating 1-2 additional studies for each cancer type.

**Results:** We report gene-based association results for rare protein-coding variants in TP53 for each of the five cancer types. We also report OR point estimates and confidence intervals for pathogenic, VUS, and benign variants in each cancer. For pathogenic variants, we conducted a meta-analysis incorporating 1-2 additional studies for each cancer type.

**Conclusion:** Our results provide evidence that rare TP53 protein-coding variants confer an increased risk of colorectal, melanoma, ovarian, pancreatic, and prostate cancer, in part due to missense variation which is not currently classified as pathogenic. Our meta-analysis results provide precise estimates of the risk conferred by pathogenic TP53 variants for each cancer type. Future studies will expand this analysis to other cohorts and integrate this dataset with additional controls from other sources, such as UK Biobank, to improve risk estimate precision.

**Keywords:** Rare, TP53, Case-control, Whole-exome, meta-analysis

CP RTP Summer Research Experience

## Abstract 44-B

The Community Scientist Program: Utilizing a multi-site rapid feedback approach to facilitate community engagement in research

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**Background:** Patient and community engagement is increasingly recognized as critical for enhancing the relevance, quality, and benefits of research. Nevertheless, factors related to time, competing priorities, and skills in interacting with communities may interfere with researchers' ability and/or willingness to take part in meaningful engagement. First established in 2018, the Community Scientist Program (CSP) aims to facilitate engagement, and improve the implementation and application of research by providing researchers with rapid feedback from trained patients and community members in order to improve the implementation and application of research within communities. This project evaluated the CSs and Researchers' satisfaction, CSP's impact on the implementation and application of research, and its effects on the Community Scientists' overall perception of research.

**Methods:** CSP has expanded to include four research institutions across geographic regions of Texas (Houston, Northeast Texas, Rio Grande Valley), encompassing both urban and rural settings. The CSP offers researchers one-hour facilitated online sessions where researchers present topics for feedback, ranging from conceptions of a research question to study implementation and dissemination to Community Scientists (CSs). Feedback forms are then sent to the researcher and the CS to gauge their satisfaction with each of the feedback sessions. REDCap was utilized to analyze saved information and analyze the effectiveness of the CSP on the benefit of research for the researchers, and its overall effect on community engagement.

**Results:** In total, the CSP has hosted 79 feedback sessions with CS across the three regions. The evaluation results revealed high researcher satisfaction with 76% of researchers feeling very prepared to engage with community members and patients following the session compared to 26% beforehand. Furthermore, 99% of Community Scientists agreed that their feedback was valued, with 100% of researchers' indicating that they would recommend the Community Scientist Program to a colleague. Furthermore, 98% of the Community Scientist agreed that the feedback they provided will help improve the research project.

**Conclusion:** Programs such as the CSP that connect researchers with the community and patient stakeholders offer a vital and scalable resource to efficiently incorporate community input and values into the research process. CSP offers a vital and scalable resource to incorporate community input and values into the research process. CSP creates a safe space where researchers and community members and patients are able to learn from one another while ensuring that the individuals who are most impacted by the research being conducted are at the forefront.

**Keywords:** Community Scientist, community engagement, patient engagement, translational research.

CP RTP Summer Research Experience

## First Year Medical Student Program

Abstract Number: 45

### **HXR9 Inhibits the HOX-PBX Cluster, Inducing Glioma Apoptosis and Cell-Cycle Arrest**

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**Background:** Gliomas are the most common malignant primary brain tumors in humans, representing over 80% of all brain cancers. Among the various molecular alterations reported in gliomas, deficiency in alpha-thalassemia/mental retardation, X-linked (ATRX), a member of the SWI/SNF family of chromatin regulators, is found in 86% of IDH-mutant low-grade gliomas (LGGs). ATRX is associated with alternative lengthening of telomeres and an aggressive phenotype. Our integrated chromatin analysis has identified HOXA cluster genes to be overexpressed in ATRX-deficient gliomas. HOXA is a developmentally essential gene, and its overexpression has been associated with an oncogenic phenotype in various types of cancers. The clinically applicable peptide HXR9 inhibits heterodimerization of HOX with PBX; therefore, we sought to determine whether HXR9 had anti-glioma effects.

**Methods:** Methods: The TCGA database was mined to identify the HOXA gene cluster expression level in LGGs and the respective survivability. Patient-derived glioma stem-cells (GSCs), GS522 (IDH1-mutant, ATRX-loss) and TS603 (IDH1-wild type, ATRX intact) were treated with HXR9 and flow cytometry was run after 48 hours of incubation to detect apoptosis (annexin V), necrosis (PI), and cell proliferation (DAPI).

**Results:** The TCGA data showed an increased HOXA expression in ATRX deficient gliomas with its role in poor prognosis. We found a dose-dependent increase in apoptosis upon HXR9 treatment, preferentially in ATRX deficient cells. Notably, our cell proliferation study suggested a decrease in S-phase of cell cycle.

**Conclusion:** With this background, we aim to further characterize the functional role of HXR9 as a therapeutic peptide for ATRX-deficient gliomas and to translate our study in preclinical in vivo models.

**Keywords:** HXR9, Glioma, ATRX, HOX

First Year Medical Student Program

Abstract Number: 46

### **Ephrin mimics may reduce metastasis and angiogenesis in breast and ovarian cancers**

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**Background:** Breast cancer is the second most common cause of cancer-related death in women, and ovarian cancer, while less common, is associated with very poor prognosis due to late-stage detection. Both cancers exhibit upregulation of Eph receptor tyrosine kinases, which are involved in cell migration, adhesion, and proliferation, and angiogenesis. This phenomenon results in aggressive cancers with high degrees of vascularization. The ligand-receptor association occurs between adjacent cells and results in "forward signaling" in the Eph-expressing cell and "reverse-signaling" in the ligand, or ephrin, expressing cell. Competitive inhibition with non-cell bound, high affinity ephrin mimics should decrease reverse signaling and cause a shift away from metastasis and angiogenesis. Because cells which undergo pathologic epithelial to mesenchymal transition (EMT) have increased metastatic capacity, the reversal of these expression levels would be an indication of decreased metastatic potential.

**Methods:** Peptide B and T are ephrin mimics, and peptide Y is a natural ligand for Eph. Novel peptides based on peptide Y were synthesized and underwent purification and analysis via prep-HPLC and LC-MS. 4T1 (breast cancer) cells were incubated with peptides B and T before Western blotting and collection of qualitative and semi-quantitative results. Synthesis of modified peptide Y primarily resulted in peptides with equal MW, as well as a peptide (peptide D) with decreased MW resulting from truncation. Eph knockout SK-OV-3 (ovarian cancer) cells and SK-OV-3 control cells were incubated with fluorescently-conjugated peptide Y. Cell lines A2780 (ovarian cancer) and control SK-OV-3 were incubated with unconjugated peptide Y (an ephrin-mimic peptide), "blocked" peptide Y, and the fluorescently-conjugated peptide D.

**Results:** Eph knockout in SK-OV-3 caused lack of peptide Y internalization as compared to the control SK-OV-3. This was enough to eliminate peptide Y binding, demonstrating the significance of Eph RTKs on ephrin binding capacity. In A2780 and control SK-OV-3, cells treated with peptide Y had high intracellular uptake, while those blocked or treated with peptide D had minimal to no uptake.

**Conclusion:** Immunocytochemistry analysis results were significant for increased E-cadherin and decreased Twist following incubation with peptide D, demonstrating potential for reduction of reverse signaling by inhibiting natural ligand binding. Confocal microscopy results indicating effective binding of peptide Y but decreased binding after blocking and a similar decrease for cells treated with peptide D demonstrate the impact of slight changes on peptide Y AA sequence on its binding capacity. Therefore, ephrin mimic peptides provide potential for decreased angiogenic and metastatic capacity in breast and ovarian cancers.

**Keywords:** breast cancer, ovarian cancer, peptide synthesis, bi-directional signaling

First Year Medical Student Program

Abstract Number: 47

## **Role of PARP inhibitors, SGLT2 inhibitors and Colchicine in Regulating Post-Chemotherapeutic Cardiotoxicity**

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**Background:** As evidenced by previous work, the Persistent Senescence-Induced Phenotype caused by mitochondrial DNA damage is implicated in post-irradiation and chemotherapeutic cardiotoxicity in cancer patients. We are investigating whether certain inhibitors (Olaparib, SGLT2 inhibitors, and colchicine) can inhibit mitochondrial DNA/telomere damage.

**Methods:** HeLa cells were cultivated until 80~100% confluency from frozen stocks in DMEM, penicillin-streptomycin, and 10% FBS. The cells were then passaged into 6-cm plates equally, and cultured until 90~100% confluence. The cells were pre-treated with Olaparib (10 $\mu$ M). 1 hour after pre-treatment, the cells were treated with Doxorubicin at 1  $\mu$ M for either 10 minutes, 30 minutes or 24 hours, and were then lysed with RIPA+ buffer with phosphatase inhibitors to collect protein. The protein lysates were spun down at 7000 RPM for 10 minutes at 4°C to isolate the protein lysate. The lysates were then transferred to a different Eppendorf tube and stored at -80°C until quantification.

**Western Blotting:** Protein quantified using the BCA Assay and BSA standards. The samples were loaded at 30 $\mu$ g/well and then run on a 10% SDS-PAGE gel. The blot was then transferred at 90V for 1 hour to a nitrocellulose membrane, blocked in 5% powdered milk in TBST, and then probed with antibodies to p-p90RSK, p-PKC $\zeta$ , and p-ERK4-S496 at a concentration of 1:1000, 1:3000, and 1:1000 respectively. The blots were then washed with TBST, incubated in goat-produced anti-mouse or rabbit IgG, at a concentration of 1:3000 and then imaged.

**Results:** We are looking at the role of p-PKC $\zeta$  in cancer cells. We wanted to see whether Olaparib (PARP inhibitor) would inhibit phosphorylation of PKC $\zeta$  or increase it. After 24 hours of treatment with Doxorubicin after 1 hour of inhibitor treatment, we observed an increase PKC $\zeta$  phosphorylation in HeLa cells, indicating the presence of chemotherapeutic mtDNA damage. The band of HeLa cells treated with doxorubicin for 10 and 30 minutes shows slightly more phosphorylation at 30 min vs 10 min. The same trend is true in HeLa cells treated with Doxorubicin for 10 and 30 minutes. However, Olaparib induction for 1 hour and doxorubicin treatment for 24 hours shows significant reduction in P-PKC $\zeta$ , indicating that Olaparib inhibits phosphorylation of PKC $\zeta$ . These results indicate that by Olaparib may be able to act prophylactically against chemotherapy-induced cardiotoxicity.

**Conclusion:** Seen inhibition of phosphorylation of PKC $\zeta$  in HeLa cells treated with doxorubicin after 1 hour of stimulation with Olaparib. This indicates that Olaparib has therapeutic potential in reducing post-chemotherapeutic atherosclerosis.

**Keywords:** cardiotoxicity, chemotherapy, colchicine, SGLT2 inhibitor, olaparib, premature senescence-induced phenotype

First Year Medical Student Program



**A preliminary study of potential variations in toxicities from RT in early stage breast Cancer patients treated pre and post-COVID with attention to ultrahypofractionation**

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**Background:** During the weeks and months after COVID, when healthcare resources were scarce, then recently published UK-FAST-forward data and the 26 Gy in 5 fraction regimen became a frequent option for early stage patients. This regimen balanced tumor control that is non-inferior to hypofractionation with decreased time in the hospital at a time when resources were short due to the COVID pandemic. Anecdotally, patients appeared to have more breast toxicity during COVID, and there was uncertainty whether this was related to COVID exposure among treated patients versus increasing use of ultrahypofractionation. This project sought to explore differences in toxicity for early stage breast cancer patients treated pre and post COVID, with attention to fractionation. The weeklong FAST-forward (ultra-hypofractionation) is being rapidly adopted in countries like UK and SWEDEN. However, in USA standard hypofractionation over 3-4 weeks remains the most frequent treatment. Hesitation to utilize the FAST-forward regimen more frequently includes the short follow-up (5 years), as well as concerns regarding toxicity. This is a preliminary report on differences seen in toxicity for ultra-hypofractionation versus hypofractionation in the pre and post the COVID era to further understand the impact of fractionation schedule and COVID exposure on breast radiation toxicity.

**Methods:** We sought to assess the impact of COVID and implementation of ultrahypofractionation on acute and 6 month toxicity radiation therapy in patients undergoing treatment for early stage breast cancer. We reviewed 299 charts from patients treated from January 2019 date to September 2021 treated with whole breast radiation with or without treatment to the low axilla. We excluded patients receiving treatment to the partial breast and patients receiving comprehensive nodal radiation. Demographic and treatment characteristics were extracted from the electronic medical record. We extracted the following characteristics: age, race, histology, T and N stage, modality, dermatitis, edema, fibrosis, breast shrinkage, breast discoloration, breast pain, ECOG, chemotherapy, hormone therapy, smoking history, cardiovascular diseases, COVID exposure, and COVID immunizations. We tabulated baseline patient and pathologic characteristics for all patients stratified by time and fractionation, hypofractionation prior to the pandemic, hypofractionation during the pandemic and ultrahypofractionation during the pandemic. 57 patients received up to 10 fractions of whole breast radiation, 243 patients received 11-28 fractions of radiation. 17 patients tested covid positive prior to radiation. 36 patients tested positive following radiation. 36 patients received COVID vaccination. Patients treated in the pre-COVID era with hypofractionation (107 patients), post-COVID with hypofractionation (141 patients), and post-COVID with ultrahypofraction (52 patients) were compared. Acute toxicity measured at the end of radiation included pain and dermatitis. Toxicity measured at the 6 month time point and later included breast edema, breast shrinkage, breast discoloration, breast pain, fibrosis. Oncora was used to identify patients with early stage breast cancer treated with lumpectomy and radiation over the designated time defined in an IRB approved protocol. Patient and tumor characteristics, as well as systemic therapy detail and dosimetric data were gathered from the EPIC EMR. We used RedCap to record and verify the data. Statistical analysis was performed in SPSS

**Results:** Using Fischer Exact test, the only toxicity endpoint with a difference between groups was acute dermatitis at the end of treatment. Comparison of patients treated with hypofractionation versus ultrahypofractionation showed a marked decrease in acute dermatitis with ultrahypofractionation ( $p < 0.001$ ). There was a trend towards increased acute dermatitis in patients treated with hypofractionation post-COVID as compared to pre-COVID ( $p = 0.06$ ). There was no difference in breast

shrinkage, breast discoloration, breast edema, or breast pain at 6 months between pre-COVID and post-COVID hypofractionation or between hypofractionation and ultrahypofractionation.

Conclusion: As expected hypofractionation was associated with increased acute dermatitis as compared to ultrahypofractionation, which is consistent with the current literature. The finding of increased dermatitis in the post-COVID era is novel, though the lack of increased toxicity at the 6 month mark does not suggest that any COVID related changes in acute breast radiation toxicity will impact long term toxicity outcomes. A lack of difference in 6 month toxicity outcomes counters the anecdotal observation of ultrahypofractionated patients experiencing increased toxicity and supports the continued routine use of this new regimen in appropriately selected patients.

Keywords: Ultra-hypofractionation, Hypofractionation, Radiation, Adverse, Effect

First Year Medical Student Program

Abstract Number: 49

**A preliminary study of potential variations in toxicities from RT in early stage breast Cancer patients treated pre and post-COVID with attention to ultra-hypofractionation**

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**Background:** During the weeks and months after COVID, when healthcare resources were scarce, then recently published UK-FAST-forward data and the 26 Gy in 5 fraction regimen became a frequent option for early stage patients. This regimen balanced tumor control that is non-inferior to hypofractionation with decreased time in the hospital at a time when resources were short due to the COVID pandemic. Anecdotally, patients appeared to have more breast toxicity during COVID, and there was uncertainty whether this was related to COVID exposure among treated patients versus increasing use of ultrahypofractionation. This project sought to explore differences in toxicity for early stage breast cancer patients treated pre and post COVID, with attention to fractionation. The weeklong FAST-forward (ultra-hypofractionation) is being rapidly adopted in countries like UK and SWEDEN. However, in USA standard hypofractionation over 3-4 weeks remains the most frequent treatment. Hesitation to utilize the FAST-forward regimen more frequently includes the short follow-up (5 years), as well as concerns regarding toxicity. This is a preliminary report on differences seen in toxicity for ultra-hypofractionation versus hypofractionation in the pre and post the COVID era to further understand the impact of fractionation schedule and COVID exposure on breast radiation toxicity.

**Methods:** Oncora was used to identify patients with early stage breast cancer treated with lumpectomy and radiation over the designated time defined in an IRB approved protocol. Patient and tumor characteristics, as well as systemic therapy detail and dosimetric data were gathered from the EPIC EMR. We used RedCap to record and verify the data. Statistical analysis was performed in SPSS

**Results:** Using Fischer Exact test, the only toxicity endpoint with a difference between groups was acute dermatitis at the end of treatment. Comparison of patients treated with hypofractionation versus ultrahypofractionation showed a marked decrease in acute dermatitis with ultrahypofractionation ( $p < 0.001$ ). There was a trend towards increased acute dermatitis in patients treated with hypofractionation post-COVID as compared to pre-COVID ( $p = 0.06$ ). There was no difference in breast shrinkage, breast discoloration, breast edema, or breast pain at 6 months between pre-COVID and post-COVID hypofractionation or between hypofractionation and ultrahypofractionation.

**Conclusion:** As expected hypofractionation was associated with increased acute dermatitis as compared to ultrahypofractionation, which is consistent with the current literature. The finding of increased dermatitis in the post-COVID era is novel, though the lack of increased toxicity at the 6 month mark does not suggest that any COVID related changes in acute breast radiation toxicity will impact long term toxicity outcomes. A lack of difference in 6 month toxicity outcomes counters the anecdotal observation of ultrahypofractionated patients experiencing increased toxicity and supports the continued routine use of this new regimen in appropriately selected patients.

**Keywords:** Ultra-hypofractionation, Hypofractionation, Radiation, Adverse, Effect

First Year Medical Student Program

Abstract Number: 50

### **Longitudinal Analysis of Lymphedema-Related Quality of Life Among Locally Advanced Breast Cancer Patients**

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Breast cancer-related lymphedema (BCRL) is a chronic and progressive, debilitating side effect of breast cancer treatment that typically occurs after axillary lymph node dissection (ALND) and/or regional node irradiation. Lymphedema is typically diagnosed at a stage where reversal of symptoms is not possible. This study aimed to find early predictors of lymphedema, with the ultimate goal of identifying an early timepoint for preventative intervention.

Women undergoing definitive treatment for locally advanced breast cancer that were treated with surgery followed by adjuvant radiation were included in this study. Patients were evaluated prior to surgery, prior to radiation, and at 6 months, 12 months, and 18 months after radiation. To measure lymphedema several objective lymphedema assessment measures were utilized. These include perometry measurements, which are volumetric measurements of the patient's arm compared to baseline, and near-infrared fluorescence imaging of lymphatics, which enable one to visualize fluid backflow in arm lymphatics using extravascular dye. We also measured several patient-reported outcomes (PROs), as patient-reported lymphedema has shown to be strongly associated with development of lymphedema related symptoms. These included EQ-5D-5L, Quick DASH, Lymphedema Symptom Intensity and Distress Survey Arm v2.0, Patient Lymphedema Symptom Survey Instrument, and Work Productivity and Impairment Survey. To evaluate the correlation between radiation dose to lymphatic structures and the development of lymphedema, we contoured the regional lymph nodes, supraclavicular, internal mammary, and axillary levels I-III since these regions drain breast cancers and are typically targeted in radiation treatment. The maximum dose, minimum dose, dose covering 95% of the structure, and percent of the volume covered by 90% of the target dose were recorded.

Analyses are ongoing to assess the baseline distribution of characteristics of patients on the study, as well as the distribution of objective and patient reported outcome measures, in addition to radiation-specific predictors of lymphedema. Our goal is to assess whether any dosimetric variables or PROs can predict for the development of lymphedema.

Conclusion:

Keywords: Breast Cancer-Related Lymphedema, Patient-Reported Outcome Measures, Axillary lymph node dissection, Near-Infrared Fluorescence Imaging

First Year Medical Student Program

### **Segmental Tracheal Resection in Advanced Thyroid Cancer Patients**

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**Background:** Less than 1% of advanced thyroid cancer (TC) patients require segmental tracheal resection. This procedure is amongst the most technically challenging and high risk in thyroid and neck surgery. Ishihara and colleagues provided the first significant experience with segmental tracheal resection for 60 advanced TC patients from 1973-1988, and this remains the largest experience in the published literature to date. Herein, we describe a contemporary high-volume experience with segmental tracheal resection for advanced TC, evaluating perioperative outcomes and survival.

**Methods:** We retrospectively reviewed consecutive patients undergoing TC surgery at MD Anderson Cancer Center from 2016-2022. Patients who underwent segmental or window tracheal resection were included, while patients with shave excisions were excluded. Primary endpoints were overall and progression free survival.

**Results:** Fifty-three patients underwent tracheal resection for intraluminally-invasive TC over a 6-year period (51 segmental; 2 window). Pathology included 37 (70%) patients with papillary TC, 7 (13%) Hurthle cell cancer, 1 (2%) medullary cancer, and 8 (15%) poorly differentiated or anaplastic thyroid cancer (ATC). Eighteen (34%) patients had a preoperative unilateral vocal cord paralysis. Median number of tracheal rings resected was 5 (range 1-12). Median hospitalization was 5 days (range 2-36). Seven (13%) patients had a new tracheostomy placed intra/postoperatively (11% temporary; 2% permanent), 4 (8%) had concomitant laryngectomy, and 24 (45%) had concomitant esophageal muscularis resection. Postoperative complications included hypoparathyroidism (40% temporary; 6% permanent), obstructive airway event requiring intervention (9%), air leak (9%), tracheoesophageal fistula (6%), wound infection (4%), hematoma (4%), pneumothorax/pneumoperitoneum (4%), and partial dehiscence of tracheal anastomosis (2%), with 11 (21%) requiring return to the operating room for management. One (2%) died within 30 days of surgery due to respiratory distress. One-year, 3-year, and 5-year overall survivals were 94%, 88%, and 88%, respectively. Median follow-up was 2.3 years (range 11 days-6 years), with 5 patients having died, 3 of which had ATC. Five (9%) patients developed locoregionally recurrent disease, all in the central neck. One-year, 3-year, and 5-year local recurrence free survivals were 92%, 80%, and 74% respectively. One-year, 3-year, and 5-year local/distant recurrence and/or progression free survivals were 68%, 44%, and 24%, respectively.

**Conclusion:** In the largest contemporary experience with segmental tracheal resection for advanced TC, we highlight the complexity and significant perioperative risks associated with these surgeries, with few patients requiring return to the operating room, and rare perioperative death. Almost all patients remain alive and locoregionally disease-free several years following surgery.

**Keywords:** segmental tracheal resection, thyroid cancer, survival complications

First Year Medical Student Program

Abstract Number: 52

### **Mechanism-rooted therapeutic strategies for inflammatory arthritis induced by checkpoint inhibitors**

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**Background:** Immune checkpoint inhibitor (ICI) therapy is a revolutionary cancer treatment that reinvigorates T cells to eradicate tumors. However, inflammatory toxicities called immune-related adverse events (irAEs) frequently occur and can affect nearly every organ system. Arthritis is the most common rheumatologic irAE and is treated with steroids but occasionally requires the cessation of ICI therapy. Given the chronicity of arthritis, balancing autoimmunity and antitumor immunity is critical in clinical management. Our lab recently analyzed synovial fluid from cancer patients with arthritis-irAE and found that T helper (Th)1, Th1-17, and Th17 cells are involved in the inflammatory process (Nat. Com. (2022)). Notably, combination ICI therapy (anti-CTLA-4 and anti-PD-1) is associated with greater steroid resistance and increased Th1-17 and Th17 cell population compared to monotherapy (anti-PD-1 alone). Currently, we lack further understanding of pathophysiology due to the lack of a preclinical murine model.

**Methods:** C57BL/6 mice were immunized with chicken collagen II emulsified with complete Freund's adjuvant (CII/CFA) along with injection PBS, anti-PD-1 antibody, or combined ICIs, and arthritis severity was later assessed. In some experiments, mice were treated with blocking antibodies against Th-17-related cytokines. At the endpoint of the experiment, we harvested spleen and bone from euthanized mice to perform flow cytometry (spleen) and histology (bone) analyses.

**Results:** Mice receiving CII/CFA developed inflammatory arthritis only after they were challenged with ICIs, recapitulating human clinical setting. Furthermore, similarly to arthritis-irAE patients, combined ICI treatment resulted in greater arthritis severity and enhancement of IL-17-producing T cells when compared to monotherapy. Importantly, blockade of Th17-related factors improved arthritis severity.

**Conclusion:** We have successfully developed the murine model of arthritis-irAE supported by clinical settings and immune landscapes similar to humans. Along with findings in patients, our mouse model lends evidence to the theory that Th17 cells may play an essential role in the pathogenesis of arthritis-irAE and that targeting factors related to these cells may treat arthritis while preserving anti-tumor immunity.

**Keywords:** Inflammation, arthritis, immune-related adverse events, checkpoint inhibitor, Th17 cells

First Year Medical Student Program

## **Golgi Apparatus in Airway Secretory Cells**

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**Background:** Mucus plays an important role in trapping foreign pathogens in the respiratory system. Mucus is formed through hydration of large, heavily glycosylated proteins called mucins, such as MUC5AC and MUC5B, along with other solutes such as salts. Mucin overproduction plays a key role in muco-obstructive diseases such as COPD. Preliminary data show that Golgi in airway epithelial cells appear dispersed. The Golgi stacks which appears separate from the classical perinuclear ribbon are called outposts. We sought to elucidate how mucin is trafficked within the cell and packaged in the Golgi apparatus using immunofluorescence (IF) and electron microscopy (EM). Understanding mucin trafficking could offer therapeutic targets in the future to halt deleterious mucus production.

**Methods:** Naïve and IL-13 treated mouse lungs were studied using IF and EM. IL-13 was used to increase mucin production. For IF, mice and human lungs were fixed in 4% paraformaldehyde and subsequently paraffin embedded. Tissue samples were cut perpendicular to major airways in 5 µm sections and stained with Golgi antibodies. Images were taken with the Deltavision Deconvolution microscope. For EM, mice lungs were harvested and fixed in 2.5% glutaraldehyde with a post-fixation in OsO<sub>4</sub>. The lung was then sectioned with a transverse cut and embedded in epoxy resin. Sections of 100nm were viewed on a Tecnai 12 transmission EM.

**Results:** Within airway secretory cells, the Golgi apparatus show a different organization compared to the classical perinuclear Golgi. For one, the perinuclear Golgi ribbon in secretory cells is extremely long, and secondly, the Golgi appears to stretch farther out towards the apex of the cell and along the lateral cell walls as shown by the NtO/NtA and CtO/CtL values in both naïve and metaplastic datasets. In addition, evidence of small individual cisternae stacks in EM along with distinct punctate in IF imply the presence of several Golgi outposts throughout the cell.

**Conclusion:** These observations of an unusual Golgi apparatus architecture can suggest many possible things. One of which could be that the need for a high volume of glycosylated protein in the form of mucin would dictate the need for a larger and more robust Golgi machinery. Another possibility is that the large size of the mucin protein (greater than 10 MDa) would mean trafficking mucin throughout the cell is impractical, thus requiring the need to synthesize and glycosylate the mucins in place before exocytosis.

**Keywords:** mucin, Golgi, Golgi outposts

First Year Medical Student Program

Abstract Number: 54

## **The mTOR Pathway Independent Function of NPRL2 in the Regulation of S-Phase DNA Damage Response**

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**Background:** Mismatch repair (MMR) was one of the first pan-cancer biomarkers for immune checkpoint blockade. However, not all tumors exhibit high mutation load due to MMR deficiency. Therefore we sought to identify if other defects in genome maintenance mechanisms could predict immune responsiveness in tumors. The GATOR1 complex functions as a negative regulator of the mammalian target of rapamycin complex 1 (mTORC1). NPRL2 is a subunit of GATOR1, and its function in the regulation of S-DDR has not yet been reported.

**Methods:** We initially conducted a genetic screen to systematically identify molecular determinants of S-phase DNA damage response (S-DDR). Once we identified that NPRL2 was a strong candidate, we used a reverse phase protein array to identify which proteins might be regulated by NPRL2. Then we used immunoprecipitation and mass spectrometry analysis to systematically identify NPRL2 interacting proteins. Then we analyzed orthotopic tumors to confirm the activation of the STING pathway and immune response to verify the therapeutic efficacy of anti-PDL1 treatment in NPRL2-deficient tumors.

**Results:** The data suggests that NPRL2 binds to an E3-ligase UHRF1 and controls UHRF1-mediated ubiquitination of CHK1 which is a key DNA damage checkpoint kinase. Loss of NPRL2 increases CHK1 protein instability which results in defective S-DDR. DNA replication, recombination, and repair and cell cycle were among the top networks of proteins interacting with NPRL2, consistent with its role in S-DDR. The findings demonstrate that NPRL2 deficiency promotes an innate immune response by the activation of the DNA sensor STING-mediated pathway.

**Conclusion:** This project identifies NPRL2 as a key regulator of genome maintenance and anti-tumor immunity. NPRL2 regulates S-DDR in an mTOR pathway-independent manner. We found that NPRL2 is required for S-DDR through its ability to regulate CHK1 degradation mediated by the UHRF1 E3-ligase. We also demonstrated that defective S-DDR in NPRL2-deficient tumors links accumulation of endogenous DNA damage to the promotion of innate immune response and tumor infiltrating lymphocytes through the activation of the STING pathway. The data provides an example for a future pathway-based biomarker to identify tumors with enhanced innate immunity, and thus predict therapeutic response to immune checkpoint blockade, particularly for tumors with low mutation load.

**Keywords:** DNA Damage Response, Innate Immunity, Biomarker, Immune Checkpoint Blockade, Genome Maintenance

First Year Medical Student Program



**Characterization of Glioblastoma Tumor Microenvironment in Humanized Mice**

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**Background:** Humanized mice are immunodeficient mice engrafted with hematopoietic stem cells (HSCs) which equips them with a reconstituted functional immune system capable of producing T and B cell-dependent immune responses, antibodies, and antiviral responses. The humanized mice model provides us with the best translational research model for investigating certain immunotherapies as treatment for glioblastomas. We want to use humanized mice as a form of targeted therapy for glioblastoma treatment by using the humanized mice model to simulate glioblastoma tumor biology, determine the efficacy of different combinations of immunotherapies on a patient's tumor, and determine the human immune response expected based on these therapies. To begin utilizing humanized mice as a form of targeted therapy in glioblastoma treatment, we have documented the tumor microenvironment of glioblastoma in humanized mice after engraftment with patient-derived xenografts (PDXs) of glioblastoma.

**Methods:** Through immunofluorescence, we were able to visualize immune cells in the tumor microenvironment by using anti-human and anti-mouse antibodies to document the humanization process using confocal fluorescence microscopy. We generated fluorescent microscopic images of immune cells in the humanized mice.

**Results:** We expect to see strong florescent signal for the human immune cells in the fluorescent images which would confirm the humanization of the mice. We used 4',6-diamidino-2-phenylindole (DAPI) to identify the nucleus of the cells in the brain tissue and different fluorophore spectra to detect human and animal immune cells. We observed a greater fluorescent signal for the human immune cells in comparison to the mouse immune cells.

**Conclusion:** We observed a greater signal for the human immune cells in comparison to the mouse immune cells indicating that the engraftment worked.

Humanized mice, glioblastoma, immunofluorescence, patient-derived xenografts, immunotherapy

First Year Medical Student

## High School Summer Program

Abstract Number: 55

### **Delineating Structure-Function Relationships in FANCA Using Chaperones**

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**Background:** The FANCA protein is essential to the tumor suppressive Fanconi Anemia (FA) pathway that protects the human genome from DNA damage. Heat shock chaperone proteins, HSP70 and HSP90, are endogenous cellular systems that reveal the severity of FANCA missense mutations. Specifically, FANCA mutants with severe cellular phenotypes have greater chaperone binding compared to other mutants. However, the exact mechanism for this enriched binding and whether these chaperone-engaging mutants have other structural or functional consequences is still poorly understood.

**Methods:** The LUMIER assay is a high throughput co-immunoprecipitation experiment that was used to quantify the interaction of mutant FANCA proteins with HSP70, HSP90, and other FA partner proteins. The ChaperISM program was used to measure the effect of FANCA mutations on HSP70 binding motifs. FANCA mutants were classified into three groups based on their predicted effects on HSP70 binding motifs: increases, decreases, or no change. Furthermore, to understand structural effects, the FoldX Protein Design Algorithm was used to predict the ddG values of FANCA mutants, with positive values representing a destabilization. Lastly, a mixed methods pipeline was developed to predict the location of FANCA interfaces with partner proteins.

**Results:** There was a poor correlation between predicted HSP70 binding groups and experimental HSP70 binding for FANCA missense mutations. However, examining the spatial distribution of HSP70 engaging mutants showed mutations that disrupt the FANCA core have more HSP70 binding than those at the surface. Moreover, many FANCA structural mutations had enriched chaperone binding. Switching focus, mutations that disrupt partner protein binding were distributed throughout the FANCA structure and not localized to interface regions. Expectedly, HSP70 engaging FANCA mutations tended to suffer the most significant partner protein binding loss.

**Conclusion:** Chaperone binding reflects the severity of FANCA missense mutations, with severe mutations having the most substantial binding. Changes in the number, quality or size of predicted HSP70 binding sites was not found to explain the chaperone binding pattern of FANCA mutants. However, predicted structural FANCA mutants showed enriched HSP70 binding and may have loss of partner protein binding.

**Keywords:** Fanconi Anemia, HSP70, Cancer, Missense Mutations

High School Summer Program

Abstract Number: 56

**Primary Breast Sarcoma: A Retrospective Single Institution Study of Clinicopathologic Features, Treatment and Prognosis**

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**Background:** Primary breast sarcomas (PBS) are a rare and heterogeneous group of cancers with limited research, publications and treatment algorithms. Previous PBS studies showed that the median overall survival (OS, time from surgery to death from any cause) for patients was 108 months. Reports using SEER data cannot report on many of the clinicopathologic features of the tumor that may affect outcome, such as surgical margins, and treatment specifics. Single institution studies have been performed; however, these have been small due to the rarity of these malignancies. In this study we aim to determine factors associated with the survival of primary breast sarcoma patients and to develop a database of primary breast sarcoma cases from a single institution.

**Methods:** We retrospectively reviewed data on 62 patients who underwent surgical treatment for breast sarcoma at MD Anderson Cancer Center from 2000-2020 that were identified from a previous institutional study. This study was approved by the MDACC Institutional Review Board (IRB). Clinicopathologic factors examined included but were not limited to patient demographics, clinical features, and pathological features. Of the patients studied, 34 had PBS, and 28 had radiation-induced sarcoma from a previously treated breast carcinoma. Prognostic factors were determined using univariable and multivariable Cox hazard ratio modeling.

**Results:** Final tumor size was the only factor significantly associated with poor prognoses for PBS patients (HR: 1.1, p: 0.03). Excisional/incisional biopsy was found to be a significant factor associated with decreased overall survival (p: 0.003), and positive margins were found to be significantly associated with increased local regional recurrence (LRR, time from surgery to first local or regional recurrence; p: 0.02); however, the data is unreliable as the confidence interval is too wide in range as a result of minimal data. No clinicopathological factors were found to be significantly associated with outcomes for breast sarcomas that were radiation-induced. There was no significant difference in OS, disease-specific survival (DSS, time from surgery to death due to breast sarcoma), or LRR based on different treatment strategies (type of surgery, neoadjuvant or adjuvant treatment). Preliminary data suggested that neoadjuvant chemotherapy was not effective in patients with PBS, except possibly in the cases of angiosarcoma.

**Conclusion:** Our data confirm that increased tumor size is associated with decreased survival for patients with PBS. Interestingly, our data also suggests that different treatment strategies were not associated with outcomes. However, more patients are needed in the database to make the data more significant due to the inherent heterogeneity of breast sarcomas.

**Keywords:** breast, sarcoma, tumor, database, retrospective

High School Summer Program

Abstract Number: 57

### **Development of Novel ATC PDX Models in Vivo**

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**Background:** Anaplastic thyroid cancer (ATC) is an extremely dangerous variation of tumors that inflicts humans with very low survival rates after a year. Although ATC makes up for a minority of thyroid cancers, it contributes to almost half of the annual mortality. In addition, ATC is usually resistant to standard chemotherapy, so patient derived xenograft (PDX) models are often used to explore possibilities with various therapeutic agents. With the close similarities in gene expression patterns and genetic differences. PDX models allow for a more accessible and effective assessment of drug responses.

**Methods:** Thyroid tumors from patients who were diagnosed with ATC prior to operations were collected and divided to develop cell lines and PDX models. In vivo mice models, the previously stated thyroid tumors were prepared into 4 x 4 x 4 mm sections and implanted into immunodeficient mice. These mice were then measured for the following weeks in order to record tumor growth volume (height x length x width). When tumors reached 1000 mm<sup>3</sup> volume, the mice were euthanized and the resulting tumor was divided into pieces to be used for PDX model expansion. PDX models are established when the mice have gone through four generations (F0 to F3).

**Results:** One ATC PDX model has been developed and suggests the possibility of many others. Two other tumors have had success growing tumors, although the volumes of said tumor have decreased since their initial growth. Many of the remaining tumors have not yet had success with growing a tumor, possibly due to their early development.

**Conclusion:** As the experiment is still ongoing, more time is needed to further expand upon developing PDX models. For the ATC PDX model that has been developed, it will undergo though profiling methods, such as hematoxylin-eosin staining, immunohistochemistry stains, and short tandem repeats DNA fingerprinting analysis, to determine the resulting model's resemblance to the original tumor.

**Keywords:** anaplastic thyroid cancer, patient-derived xenografts, short tandem repeats, hematoxylin-eosin staining

High School Summer Program

**ApoA-I binding protein (AIBP) decreases mechanical hypersensitivity after plantar incision in a rat model of post-operative pain**

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**Background:** Post-operative pain is common in surgical patients, with many reporting inadequate pain relief. This can lead to cardiopulmonary complications, delayed mobilization, longer hospitalization, sleep disturbances, and psychological stress. Pro-inflammatory cascades triggered by toll-like receptor 4 (TLR4) activation contribute to chronic pain. Specifically, TLR4 localizes to lipid rafts and dimerizes, initiating inflammatory signaling. Apolipoprotein A-I binding protein (AIBP), a secreted protein that has been shown to bind ApoA-I and high-density lipoprotein, can reduce lipid rafts by removing excess cholesterol from the plasma membrane. Because lipid rafts play a key organizational role in neuronal membranes, changes in lipid rafts can produce pro- or anti-nociceptive effects. Modified AIBP that binds to TLR4 has been shown to reduce hypersensitivity in preclinical models of inflammatory and neuropathic pain. In this study, we aim to investigate the impact of AIBP on mechanical hypersensitivity and wound healing in a post-operative pain model.

**Methods:** In this study, adult male and female Wistar rats from Harlan were used. To model post-operative pain, all rats received a 15 mm incision through the skin and fascia of the plantar hind paw. The plantaris muscle was stressed, then the wound was closed with two sutures. The rats in the AIBP treatment group were injected intravenously with 0.3 mg AIBP 30 minutes before the surgery. Von Frey filaments (0.4, 0.6, 1, 2, 4, 8, 10, and 15 g) were used to determine mechanical paw withdrawal threshold via the up-down method. Rats were tested before surgery and at 2 hr, 4 hr, 24 hr, 48 hr, 72 hr, 7 days, and 10 days after surgery. Images were taken at 3, 6, 9, and 10 days after surgery to determine wound size and rate of healing.

**Results:** Intravenous AIBP pre-treatment significantly attenuated paw incision-induced mechanical hypersensitivity in the ipsilateral hind paw compared to untreated controls in male rats. In females, there was no significant difference in mechanical hypersensitivity between the two groups, and the wound size and rate of healing between the two groups did not differ. However, additional subjects are needed for sufficient statistical power.

Conclusion: Targeting TLR4 lipid rafts with AIBP could be an effective method to reduce post-operative incision pain without compromising wound healing. Ongoing studies are investigating the potential role of AIBP on wound healing in males and the mechanisms that caused the mechanical hypersensitivity difference between males and females.

Keywords: Post-operative pain, mechanical hypersensitivity, AIBP, TLR4

High School Summer Program

### **CCR4 Antagonists in Cutaneous T-Cell Lymphoma (CTCL)**

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CC Chemokine Receptor 4 (CCR4) is highly expressed by type 2 helper T-cells (Th2) and regulatory T cells (Treg). It orchestrates cell migration and leads to the accumulation of Th2/Tregs in tumors, allowing tumors to evade immune attacks. Cutaneous T-Cell lymphoma (CTCL) cells have Th2/Treg phenotypes and highly express CCR4, which serves as a prominent therapeutic target for CTCL. We hypothesize that treatment with CCR4 antagonists will decrease CTCL cell accumulation and inhibit tumor development and progression in CTCL.

C021-dihydrochloride, a small molecule CCR4 inhibitor, was investigated in this study. NSG mice were used for xenograft mouse models by injecting MJ CTCL cells subcutaneously. Arm 1 mice received C021 and MJ cells simultaneously; Arm 2 mice were first injected with MJ cells, then treated with C021 after tumor formation. Each arm included 3 groups (5 mice/group): Group 1 – vehicle control, Group 2 – low dose (5 mg/kg), Group 3 – high dose (20 mg/kg). Tumors were removed and weighed for each group at the end. The proliferation marker Ki67 (monoclonal rabbit antibody D3B5, 1:3000) and CCR4 (polyclonal human antibody NBP1-86584, 1:500) expression in tumors were analyzed using immunohistochemistry (IHC). The average of three graders was taken to measure the level of expression for each section. Ki67 stains were graded by the percentage of positive cells. CCR4 stains were graded by the percentage of cells within 4 intensity classes: negative, weak, moderate, and strong.

Arm 1 mice had a lower average tumor weight in the low dose (56.00±42.20 mg) and high dose groups (46.60±35.18 mg) compared to the control group (153.80±95.71 mg). Lower average tumor weights were also observed in Arm 2 low dose group (164.60±101.3 mg) compared to the control group (220.50±102.50). In Arm 1 mice, the high dose group (17.83±21.43) had a lower average Ki67 score compared to the control group (28.54±10.68). The low dose group in Arm 2 had a lower average Ki67 score (11.19±11.33) than the control group (20.29±17.73). Arm 1 CCR4 expression did not have major differences between control (59.17±27.30), low dose (62.22±19.32), and high dose group (67.78±11.82). Arm 2 also did not have a major difference between control (74.58± 46.77) and low dose group (69.22±23.84).

Our results suggest that C021 treatment led to decreased tumor volume and decreased tumor proliferation. However, levels of CCR4 expression did not drastically change. Future studies will assess Th2/Treg phenotypes, apoptosis, and necrosis to elucidate the anti-tumor effects of C021 in CTCL.

Keywords: CTCL, CCR4, immunohistochemistry, Ki67, tumor proliferation

High School Summer Program

Abstract Number: 60

### **Effect of ZRANB1 on PARP inhibitor resistance in breast cancer cells**

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**Background:** When DNA is damaged, the repair process is usually activated with Poly (ADP-ribose) polymerases (PARPs). PARP is an enzyme found in our cells and its function helps damaged cells repair themselves. PARPs act as DNA damage sensors and bind to those damaged DNA at single strand DNA breaks site in which signals DNA repair effectors to the breaks sites. PARP inhibitors stop the enzymes from repairing damaged cells in cancer cells which causes the cells to die. In breast cancer cells (BRCA1/2 deficient tumor cells) are more sensitive to PARP inhibitors (PARPi) due to synthetic lethality. Since there are cancer patients who may or may not be resistant to the sensitivity of drugs, the PARP inhibitor allows us to investigate the mechanism. The aim is to learn about the mechanisms behind PARP inhibitor resistance, specifically in breast cancer cells, using the protein ZRANB1.

**Methods:** Immunoblotting of ZRANB1 and GAPDH in parental SUM149 and PARPi-resistant (SUM149-PR) cells. SUM149-PR cells stably expressing doxycycline (DOX)-inducible ZRANB1 shRNA were treated with DOX (100 ng/ml) for 4 days, followed by immunoblotting with antibodies against ZRANB1, NBS1, RAD50, MRE11, and  $\beta$ -actin. SUM149-PR cells stably expressing 764 doxycycline (DOX)-inducible ZRANB1 shRNA were treated with different concentrations of talazoparib with or without DOX for 6 days. Cell viability was measured by a CCK8 assay (left panel). Hereafter, the IC50 value was defined as the concentration of PARPi resulting in a 50% reduction in the number of drug768 treated cells compared with the number of control cells (right panel). n = 3 wells per group. Left panel: BT549 cells stably expressing doxycycline (DOX)-inducible ZRANB1 shRNA were treated with different concentrations of olaparib with or without DOX for 3 days. Cell viability was measured by a CCK8 assay. Right panel: the IC50 values. n = 3 wells per group.

**Results:** Knowing before that the protein ZRANB1 increases the PARP inhibitor. The knockdown of ZRANB1 shows its mechanism with PARP inhibitor resistance in breast cancer cells.

**Conclusion:** Based on the results, the knockdown of ZRANB1 resensitizes the resistant cells to the PARP inhibitor.

**Keywords:** PARPi, breast cancer, damaged DNA, ZRANB1, resistance

High School Summer Program



## ITERT Undergraduate Summer Research Training Program

Abstract Number: 61

### **Prevention of early stage Kras-mutant lung adenocarcinoma via targeted KRAS<sup>G12D</sup> Inhibition**

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**Background:** Our group recently identified alveolar intermediary cells (AICs) that arise early on post-tobacco carcinogen exposure *in vivo* and during AT2-mediated repair mechanisms. Notably, and prior to tumor onset, a subset of AICs harbored driver *Kras*<sup>G12D</sup> mutations which were later found in the resultant LUADs, and which comprise the same variants found in human smoker LUADs. These findings support a role for AICs as precursors for LUAD pathogenesis. Our ongoing studies are studying AICs at high resolution to identify targets for early prevention and/or interception of LUAD pathogenesis, including specifically targeting *Kras*<sup>G12D</sup> mutations. While targeted KRAS<sup>G12D</sup> inhibitors (e.g., MRTX 1133, Mirati) are showing strong pharmacological activity against frank human lung cancer, the effects of these agents on tumor precursors is not known. Evaluation of MRTX 1133 as a KRAS<sup>G12D</sup> inhibitor in preclinical mouse *in vitro* and *in vivo* models is necessary to assess its effectiveness as an intervention strategy against early pathogenesis of LUAD.

**Methods:** Here, we treated mF471 murine lung cancer cells from mice with knockout of *Gprc5a* (*Gprc5a*<sup>-/-</sup>) with varying concentrations of MRTX 1133. We then utilized an MTT proliferation assay and western blotting analysis to probe the minimal inhibitory concentration (MIC) of MRTX 1133 on cell growth as well as the effects of this drug on signaling components downstream of KRAS activation.

**Results:** We found that increasing concentrations of MRTX 1133 resulted in statistically significant decreases in cell viability when compared to control-treated cells. In addition, MRTX1133 showed dose-dependent inhibition of mitogen-activated protein kinase (MAPK) signaling at various time points tested, with significant reductions as early as 3 hours post-treatment and concentrations as low as 1 nM.

**Conclusion:** These results suggest that MRTX 1133 inhibits various malignant phenotypes (growth, pro-tumor cell signaling) of murine *Kras* mutant lung cancer cells. This study provides new insights into effectiveness of MRTX 1133 in murine lung cancer cells as a precursor to targeted clinical prevention of LUAD *in vivo*. Further experiments are underway to assess the anti-tumoral (including preventive) impact of MRTX 1133 *Kras*<sup>G12D</sup> inhibition using genetically engineered *in vivo* and *ex vivo* mouse models.

**Keywords:** Lung adenocarcinoma, *Kras*<sup>G12D</sup> inhibition, MRTX 1133, targeted prevention

ITERT Undergraduate Summer Research Training Program

## Non-Affiliated Summer Students

Abstract Number: 63

### **Tungsten nanoparticle and polymer blends as novel coating for radiopaque resorbable inferior vena cava filters**

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**Background:** Deep vein thrombosis (DVT) or pulmonary embolism (PE) affects about 900,000 individuals annually in the United States. Placement of inferior vena cava filters (IVCFs) is used for patients with contraindications to anticoagulation. However, non-retrieval of IVCFs pose patient safety concerns including risks of IVCF migration, embolization, and thrombosis. Absorbable IVCFs constructed with poly-p-dioxanone (PPDO) eliminate the need for filter retrieval, whilst improving radiolucency of PPDO-IVCFs through addition of radio-enhancing nanoparticles (NPs) such as gold (Au) and bismuth (Bi). To further improve the performance of radiopaque absorbable IVCFs, we explored the use of tungsten (W) NPs with polyhydroxybutyrate (PHB), polyvinylpyrrolidone (PVP) and polycaprolactone (PCL) polymer blends as coating materials for PPDO.

**Methods:** WNPs were synthesized by thermal decomposition method. WNPs were characterized in terms of size using dynamic light scattering and powder X-ray diffraction (XRD) patterns, morphology using transmission/scanning electron microscopy (TEM/SEM), and radiopacity using computed tomography (CT). WNPs combined with PHB, PCL, and PVP polymers were coated onto PPDO using a wet-dipping technique. In vitro biocompatibility was tested against EC-RF24 using alamarBlue assay. Rat blood was used to screen hemolytic activity upon contact with NP-coated PPDO. W+PHB and W+PHB+PCL+PVP IVCFs were deployed in separate pig models. Autologous thrombus was deployed 1-2 weeks after filter implantation. Hematological tests were monitored and images of the chest, abdomen, and pelvis were taken weekly for 12 weeks via CT imaging.

**Results:** The additional PCL+PVP coating with WNP+PHB enhanced filter visualization during fluoroscopy-guided deployment into porcine models. Moreover, initial CT monitoring showed 14-fold signal enhancement of WNP+PHB+PCL+PVP ( $1201 \text{ HU} \pm 885 \text{ HU}$ ) compared to the background (muscle area,  $62 \pm 13 \text{ HU}$ ), while WNP+PHB was only 8-fold higher ( $468 \pm 220 \text{ HU}$ ) than the background (muscle area,  $62 \pm 8 \text{ HU}$ ). Maximum HU values showed decreasing CT intensities throughout the 12 weeks for both WNP+PHB only and WNP+PHB+PCL+PVP. Although WNP+PHB+PCL+PVP showed higher intensity at week 12, gross necropsy images show necrotic lumen, suggesting local toxicity brought about by the implanted IVCF.

Conclusion: Bioresorbable IVCFs coated with WNPs and PHB+PCL+PVP reinforcement can be fully visualized with fluoroscopy and CT for real-time image-guided deployment and monitoring of IVCF in pigs. However, initial findings suggest possible local toxicity in the luminal wall brought about by implantation of WNP+PHB+PCL+PVP filter. Further analyses are currently ongoing to assess the in vivo safety of WNP+polymer-coated IVCFs, as well as the use of other high-Z nanoparticles.

Keywords: IVC filters, nanoparticles, medical device

Non-Affiliated Summer Students

Abstract Number: 63-A

## **Utilizing Patient-Reported Outcome Questionnaires in Standard Practice: A Study from Thoracic Radiation Oncology**

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**Background:** Patient-reported outcome (PRO) questionnaires are one approach to improve patient-provider communication, enhance satisfaction, and increase survival. However, there is limited research on its effectiveness in radiation oncology, and providers are hesitant to implement PROs due to possible disturbance of clinical flow or lack of utility. So, we examined provider and patient sentiments towards PRO questionnaires as part of routine clinical care.

**Methods:** Patients and providers were given a questionnaire to elicit their viewpoints on PRO utilization in the thoracic radiation oncology (TRO) department before and after PRO implementation. The provider questionnaire included questions about the benefits and downfalls of implementation. The patient questionnaire included questions about care management, communication, decision-making, and comfort level. Questionnaire answers included “always, often, sometimes, rarely, never.” We used summary statistics to compare surveys before and after PRO implementation.

**Results:** Overall, 16 providers (6 [37.5%] attendings, 6 [37.5%] midlevels, 4 [12.5%] nurses) and 45 patients completed the pre-surveys, while 16 providers (8 [50%] attendings, 5 [31.3%] midlevels, 3 [18.8%] nurses) and 96 patients completed post-surveys. In the provider pre-surveys, 44% believed that PRO-CTCAE questionnaires “somewhat improve” clinical flow. In the post-survey, 94% were neutral. In the pre-survey, “visualizing data” was selected as the main advantage of PROs compared to creating a high-alert-value system in the post-survey. In the patient pre-surveys, 93% “always” felt comfortable with providers’ symptom-related questions, compared to 73% in the post-survey. In the pre-survey, 84% “always” felt their provider communicated well, compared to 73% in the post-survey. 31% in the pre-survey agreed that they were taught coping mechanisms “very well,” which decreased to 26% in the post-survey. 81% in the pre-survey “always” agreed that they understood how to manage symptoms, which decreased to 51% in the post-survey. When asked how often patients were given information to help make decisions about managing their symptoms, 47% responded “a great deal” in the pre-survey compared to 41% in the post-survey.

**Conclusion:** Pre-implementation, providers were optimistic that PROs could improve clinical flow. However, most developed a neutral stance post-implementation, suggesting an opportunity for improvement. Providers wanted PROs for data visualization and creation of a high-alert-value system; thus, these elements should be considered in PRO implementation. Patients’ perception of patient-provider communication did not improve post-implementation. Patients desired more information on symptom management and comfort. This suggests that while PRO implementation is an important tool to determine where communication gaps exist, additional strategies are needed to improve communication.

**Keywords:** Patient-reported outcomes, Thoracic Radiation Oncology

Non-Affiliated Summer Students

## **Detecting and Intervening on Rare Pre-Existing Resistant Subclones to BRAF/MEK Inhibitors in Melanoma**

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**Background:** A challenge to cancer treatment is acquired resistance to targeted therapies. Little is known if drug resistance occurs de novo or how to best counteract them. In melanoma, patients with BRAF activating mutations are treated with BRAF and MEK inhibitors (BRAFi+MEKi). Although the majority of these patients respond to therapy, over 80% of patients progress on therapy within one year.

A majority of resistant mutations found are in the MAPK or PI3K pathways and are postulated to be the resistance mechanism. Unfortunately, targeting these resistant mutations after relapse have not proven effective, and thus demonstrate clinical unmet need.

**Methods:** We identified 147 patients with BRAF-mutated cutaneous melanoma from The University of Texas MD Anderson Cancer Center. 79 FFPE patient tumor samples from 29 patients had their tumor DNA extracted using the Covaris truXTRAC Automated FFPE Kit according to protocol with UNG treatment at 50C for 1 hour after reverse crosslinking step. A Zymo Quick-DNA Microprep kit was used to remove excess melanin to optimize PCR amplification. qBDA technology enriched several different groups from the MAPK or PI3K pathways via different BDA primer/blocker sets, and then underwent next-generation sequencing.

Patient data was obtained from EPIC, including progression-free survival (PFS) while on BRAFi+MEKi treatment, best response (RECIST), and overall survival (OS). PFS and OS Kaplan-Meier curves were generated through GraphPad Prism 9. Statistical significance was determined through Log-rank (Mantel-Cox) test using Prism software. Values were determined to be statistically significant if  $P < 0.05$ .

**Results:** Out of the 29 patients, 12 (41.4%) patients were determined to have no mutations, while 17 (58.6%) patients had detectable mutations. The median PFS of patients without any mutation was 8.45, while with any mutation, the median survival was 5.00. The p-value for the data was .137, indicating that the data is not significant. Given that this is preliminary data, the increased number of samples once the project is completed will hopefully yield significant results.

**Conclusion:** In this interim analysis, patients that did not have a detectable resistance mutation using qBDA in the MAPK/PI3K pathways had a trend towards overall higher PFS compared to the group with any mutation. This preliminary data supports the hypothesis that pre-existing mutations may confer resistance to BRAFi/MEKi therapy that leads to tumor progression. The next steps are to obtain more patient samples to demonstrate how personalized counter-resistance therapies can increase the long-term outcome of patients harboring pre-existing low-VAF resistance subclones.

**Keywords:** melanoma, BRAF, MEK, resistance, mutation

Non-Affiliated Summer Students

### **Dose Accumulation with CBCT Conversion in Head and Neck and Prostate**

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**Background:** Standard radiotherapy utilizes a single CT study to inform treatment planning, however, tumor(s) and organs at risk can shift and change size over the course of treatment, thereby rendering initial planning less effective. These changes can lead to significant dosimetric effects and suboptimal therapy if not taken into account. While daily imaging using Cone Beam CT (CBCT) can ameliorate such challenges and improve therapeutic delivery limitations that exist. Compared to CT, CBCT, image quality is lower, and the field of view is smaller leading to potential errors in the dose calculations.

**Methods:** To enable daily CBCT-based dose calculations, CBCTs were converted to synthetic CTs via deformable image registration (DIR) techniques, which resolves the spatial differences between sets of images. Planning and weekly CTs, and daily CBCTs were imported into a commercial treatment planning system (TPS) and rigid registrations and DIRs were applied. Next, CBCTs were converted to synthetic CTs using a clinical algorithm in the TPS. Doses were calculated and dose accumulation was performed

**Results:** The TPS algorithm deployed facilitated the successful conversion of CBCTs to synthetic CTs with smooth transitions. Synthetic CTs were then used to accurately determine dose calculations while taking into account daily shifts and changes in the size of tumor(s) and organs at risk. Accumulated dose calculations using converted CBCTs were compared to planned doses and yielded up to a  $\pm 10\%$  difference to organs at risk. The clinical impact of these dose differences are currently being investigated. Lastly, we determined that CBCTs can be converted successfully with either the planning CT or the weekly CT since minimal dose differences were observed between the two methods.

**Conclusion:** CBCT conversion shows promise for applications in Head and Neck, and Prostate Cancer Radiotherapy.

**Keywords:** CT, CBCT, Radiotherapy, Head and Neck, Prostate

Non-Affiliated Summer Students

## Partnership for Careers in Cancer Science and Medicine

Abstract Number: 64

### **Immunopathogenesis of *Granulibacter bethesdensis*, an opportunistic pathogen causing recurrent infection in immunocompromised Chronic Granulomatous Disease Patients**

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**Background:** Chronic Granulomatous Disease (CGD) is a X-linked genetic disorder that affects the white blood cells, specifically phagocytes leading to a delay in the ability to promptly expel any invading bacterial or fungal pathogen. Bacterial infections caused by *Granulibacter bethesdensis* (Gb) were first identified in CGD patients. Healthy individuals are unaffected by Gb infections, but CGD patients require the aid of antibiotics to clear the chronic infections caused by Gb. Infection manifests in the form of fever and necrotizing lymphadenitis which may recur or relapse. Very little is known regarding host immune responses required for efficient clearance of this pathogen in lung microenvironment, the primary site of recurrent infections in CGD patients. A murine X-CGD model of Gb infection has been developed to allow us to better understand the roles played by different immune cells during different phases of infection.

**Methods:** In this study, WT and X-CGD mice (n=13) were infected with Gb and euthanized at Day 1, Day 4, and Day 12. Mice were monitored for clinical signs of infection and upon euthanasia, blood, bronchioalveolar lavage (BAL), and lungs were collected for bacterial burden and lung tissues for histology. Cells were isolated from BAL for slide preparation to determine their phenotype and morphology using Wright-Giemsa stain.

**Results:** The WT mice displayed a transient increase in clinical score correlating with moderate increase in local and systemic bacterial burdens characteristic of a mild, resolving infection, XCGD mice exhibited a marked increase in clinical scores and bacterial burdens which remained high through Day 12 post infection, the time at which 100% of XCGD mice became moribund. Histology of lungs from WT and XCGD mice showed significant increase in white blood cell counts within the lung microenvironment. Microscopy revealed that BAL cells primarily comprised of macrophages and neutrophils with the numbers mirroring the clinical severity of infection among WT and XCGD mice. We observed high numbers of macrophages and neutrophils at Day 1 among both groups of mice with the cell numbers returning to basal levels by Day 4 among WT mice. An increase in neutrophil counts was observed in XCGD mice at Day 4 and remained high at Day 12 indicating a continued state of inflammation within the lung microenvironment.

**Conclusion:** The inability to efficiently clear Gb among XCGD mice causes an alteration to the lung microenvironment which led to an exacerbated disease condition. With the rise of multi-drug resistant bacteria, we need to focus on alternative therapeutic strategies capable of boosting the immune system while minimizing treatment costs.

**Keywords:** Chronic Granulomatous Disease, *Granulibacter bethesdensis*, Giemsa Staining, Phagocytes, Bronchioalveolar Lavage Fluids (BALF)

Partnership for Careers in Cancer Science and Medicine

### **The Effects of a Brief Educational Intervention on COVID-19 Knowledge, Beliefs, and Intention to Get Vaccinated in Black and Hispanic Populations**

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**Background:** Vaccine hesitancy is prevalent in minority populations, specifically Black and Hispanic populations in the United States due to medical mistrust stemming from historical events, and/or negative perceptions vaccines due to misinformation (Momplaisir, et al., 2021; Doherty, et al., 2021; Willis, et al., 2021; Khubchandani, et al., 2021). The use of media and technology may be crucial in disseminating pertinent and accurate information to increase vaccine acceptance among these communities. Understanding strategies to reduce vaccine hesitancy is critical to acquiring herd immunity. This study seeks to explore the role of a brief educational intervention on an individuals' knowledge of the COVID-19 vaccine, belief of its effects, and intention to receive the vaccine.

**Methods:** Data from the Community Engagement Alliance Against COVID-19 Disparities study was used. English-speaking Black and Hispanic adults were recruited for the study (n = 1606, mean age = 31.9 ± 5.9). Participants were primarily recruited from community-based organizations, and all participants were unvaccinated against COVID-19. After being screened for eligibility, consented participants completed a baseline survey, viewed the COVID-19 educational materials, and then completed a follow-up survey. The educational materials consisted of a frequently asked questions document, an infographic, a social media guide, and two ethnically appropriate social media flyers. Factor analysis was performed for vaccine knowledge and belief measures. Knowledge score was created by summing two items (score range from 0 - 2). Similarly, belief score was calculated by summing two items (score range from 0 - 2). Intention to get the COVID-19 vaccine in the next 30 days was assessed using 1 item, treated as a continuous score (score range from 1 – 5). Change in score before and after the educational intervention was assessed with paired t-tests (or Wilcoxon signed-rank test when appropriate).

**Results:** The mean scores for COVID-19 knowledge increased from 1.07 ± 0.87 to 1.29 ± 0.85 (p<0.0001), beliefs increased from 1.20 ± 0.88 to 1.36 ± 0.75 (p<0.0001), and intention to get vaccinated in the next 30 days increased from 3.18 ± 0.92 to 3.45 ± 0.88 (p<0.0001).

**Conclusion:** The educational intervention led to significant increases in COVID-19 knowledge, positive beliefs, and intention to get vaccinated in the next 30 days. These findings indicate that one strategy that may prove effective in decreasing vaccine hesitancy in Black and Hispanic populations is short, ethnically appropriate educational materials. These materials can be posted in schools, waiting areas, places of worship, the workplace, or other frequently visited areas to educate the public on the COVID-19 vaccine, increase vaccine acceptance, and lead us closer to herd immunity.

**Keywords:** COVID-19, vaccine hesitancy, Black, Hispanic

Partnership for Careers in Cancer Science and Medicine



Abstract Number: 66

### **Deciphering the multifaceted roles of a novel ultraconserved noncoding RNA, overexpressed in Chronic Lymphocytic Leukemia**

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**Background:** Chronic Lymphocytic Leukemia (CLL) is a malignancy of CD5+ B cells that primarily occurs in the blood and bone marrow. It's one of the most common blood cancers in Western countries and about 10% of patients diagnosed with CLL develop into Richter Syndrome (RS), an extremely aggressive transformation into lymphoma. These patients have a poor survival rate with a median survival of 12 months. This death sentence is the reason why we want to better understand the aggressive transformation of CLL and investigate novel factors contributing to the genetic events behind this transformation. Ultra-conserved regions (UCRs) are genomic regions that are perfectly (100%) conserved in between humans, mice and rats. Some of the UCRs get transcribed into long noncoding RNAs often called as Transcribed Ultraconserved regions. Perfect conservation of T-UCRs makes them interesting and aberrations in such regions has been correlated to cancer susceptibility. TRUC-16 is found to be overexpressed in aggressive CLL and Richter patients. Direct interaction of TRUC-16 to p16INK4A leads to lower levels of p16INK4A and overexpressed conditions of TRUC-16. P16INK4A, a tumor suppressor, is found to be silenced in 30% of Richter Transformation cases. We hypothesize that forced overexpression of TRUC-16 in B cells leads to aggressive transformation into CLL and RS-like phenotype in C57BL6 mice.

**Methods:** In order to investigate the disease phenotype in the CRISPR-Knockin mice, generated in Dr. Calin's lab, we performed flow cytometry to assess lymphocytes in the peripheral blood. The Flow cytometry analysis was performed using two panels comprising of CLL markers (CD5, B220, CD19, IgK and Igλ) and a Richter panel including CD23, CD20, and Ki-67. Retro-orbital bleedings were performed on mice to collect peripheral blood that was further subjected to surface and intracellular staining. Data was acquired using BD LSR Fortessa and further analyzed using FlowJo software.

**Results:** Our results show that native B cells are potentially migrating to spleen and lymph nodes in old mice nearing the transformation timepoint.

**Conclusion:** Further, to characterize the B cell subtype, we would design a new panel utilizing CD80, CD27, CD138, and other B cell markers to better understand the B cell population in the immune compartment of these TRUC-16 overexpressing mice. This study will help us in understanding the role of T-UCRs in CLL and RS and help in identifying the key regulators that drive this aggressive transformation

**Keywords:** CLL, Richter Syndrome, Ultraconserved

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 67

### **A Cancer Gene-Drug Connectivity Map for DrBioRight**

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**Background:** The DGldb provides gene-drug interactions that are curated by experts' manual curation and text-mining from public resources, including Drug Target Commons and DrugBank. Such interaction information can help easily identify potential drug targets or check whether a cancer related gene is targetable by any drug. By incorporating such information, our users can visualize the associated drugs for each queried gene and easily find the corresponding publication information for each gene-drug interaction.

**Methods:** First, 261 cancer genes were obtained from TCGA highly mutated cancer genes. Second, interactions (~80,000) for the 261 cancer genes were filtered from DGldb. Third, two histograms were generated to show the gene and drug association. Fourth, high frequency genes and drugs were sorted to generate the two lists by using cutoff of greater than or equal to 100 for genes and greater than or equal to 20 for drugs. Then, bar plots were created to show two lists from output of step four. 82 interactions were extracted based on the high frequency genes and drugs from step four. Lastly, data from the previous step was used to generate connectivity map using Cytoscape.

**Results:** Figure 1 shows the number of interacted drugs for each gene, in which there is one gene associated with 770 drugs. Figure 2 shows the number of genes for each interacted drug. Figure 3 and 4 summarize the top genes and drugs ranked by their degrees in the interaction network, in which, the gene NFR2L2 and the drug CETUXIMAB have the highest number of neighbors. Figure 5 shows a sub-connectivity map constructed by the top genes and drugs identified from Figure 3 and 4.

**Conclusion:** Our results serve as the foundation in building a new module for DrBioRight. It will help users obtain information of drug-gene interactions conveniently in DrBioRight rather than referencing other databases. The new module for DrBioRight will consolidate the data generated in this study to further increase the utility and improve its user experience.

**Keywords:** DrBioRight, Connectivity Map, DGldb, Cancer Gene-Drug

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 69

### **The Arginine Methylation of p14ARF**

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**Background:** P14ARF is a tumor suppressing protein that inhibits the growth of cancer cells by indirectly activating p53 which is a key tumor suppressing gene. P14ARF is mostly made up of the following four amino acids Arginine, Alanine, Glycine, and Leucine. This protein is important to deepen our understanding of cancer progression.

**Methods:** (1) express recombinant p14ARF protein via autoinduction in E. Coli expression system, (2) purify p14ARF via chromatography, and (3) characterize the posttranslational modification via arginine methylation.

**Results:** After we expressed the recombinant p14ARF protein via autoinduction we split the protein into 4 separate samples and used a different purification process for each. As it turns out the samples with the His-SUMO tag show higher methyl activation than the samples that are not His-SUMO tagged.

**Conclusion:** Sample 3 which went through the nickel column, and was concentrated, and sample 4 which went through the nickel column, then the SP column, and was concentrated showed both showed high methylation activity compared to the others. Samples 3 and 4 are His-SUMO tagged. Sample 2 is cleaved but contains both p14ARF and His-SUMO. Sample 2 has very weak activity. In the future we plan to optimize the activity of the methylation of p14ARF.

**Keywords:** P14ARF, Arginine Methylation, His-SUMO

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 70

### **Investigation of Acute Hypoxia Effects on a 3D Model of Renal Cell Carcinoma**

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**Background:** Genetic alterations of the hypoxia response pathway are common drivers of clear cell Renal Cell Carcinoma (ccRCC). On the other hand, non-clear cell Renal Cell Carcinoma (nccRCC) usually displays a genetically intact response-to-hypoxia stress signaling, suggesting a unique response of nccRCC when subjected to low oxygen levels. Understanding how different genomic driven Renal Cancers survive under hypoxic stress is of extreme importance for understanding nccRCC progression

**Methods:** We derived three-dimension tumor culture systems (tumor organoids) from explants obtained from a Genetically Engineered Mouse Model (GEMM) of nccRCC with somatic mosaic knockouts of key drivers of nccRCC evolution, already established in our lab. After organoid stabilization, they were challenged with severe hypoxia (1% hypoxia) or with normoxia conditions (21% oxygen) for 72 hours. Random fields of tumor organoids were collected at time 0 (immediately before hypoxia or normoxia treatment) and 72 hours after hypoxia or normoxia treatment. Data from images were analyzed with ImageJ software FIJI and statistical analysis with GraphPad Prism 9.

**Results:** A statistically significant ( $p < 0.05$ ) difference was observed in numbers of organoids that were challenged with normoxia, while no statistically significant difference was observed tumor organoids challenged with hypoxia after 72 hours. Likewise, size of organoids in normoxia showed statistical significance ( $p < 0.01$ ) while no differences in the organoids in hypoxia after 72 hours.

**Conclusion:** Our data suggests that hypoxia severely impacts tumor organoids proliferation and stemness capabilities of nccRCC. Further investigation is required to better characterize the effects of hypoxia on models of nccRCC: 1) prolonged hypoxia challenge (i.e 7 days) should be performed with different levels of hypoxia (i.e. 5%, 2%, 0.5%); 2) different genetic background should be challenged to hypoxia in to clarify genomic events responsible for sensitivity or resistance to low oxygen levels.

**Keywords:** Hypoxia, RCC, Tumor organoids, GEM model

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 71

### **Determining the Growth Rate of Various Escherichia coli Cell Lines in Different Broths**

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**Background:** One of several organisms of choice for producing recombinant proteins is Escherichia coli. There are a variety of E. coli cell lines that can be used for protein expression. These cell lines are often cultivated in Luria broth (LB) and Terrific broth (TB). However, there are many broths that E. coli cell lines can be cultivated in. We wanted to determine the growth rate and expression level of four protein constructs (1345, 1313, 1208 & 1211) in four E. coli cell lines utilizing various broths. In this study, we analyzed growth rates in the following cell lines: BL21 (DE3), BL21 Star 905, BL21 Star 906, and BL21 Star 907. The broths that were tested include TB, 2XY, Superior, Hyper, LB, Power, Glucose, and Autoinduction media.

**Methods:** Each cell line was cultivated in an overnight culture using LB. The overnight cultures were diluted with media to have an optical density (OD) of two. The diluted overnight cultures were transferred into 96 well plates with the appropriate media and construct. Every 30 minutes the absorbance was measured at a wavelength of 600 nm until it reached the inducible OD. IPTG was then added to the cell lines. Protein expression was analyzed using the dot blot method.

**Results:** Our approach demonstrated that the broth that produced the desired OD for each cell line the fastest was TB. The OD of the BL21 cell line was attained in 2 hours for TB, 2XY, and LB broths. The OD of the other broths was obtained after two hours and thirty minutes. The optical density of the remaining cell lines ranged between two and thirty minutes and three hours. BL21 (DE3) and BL21 Star 905 cell lines expressed the constructs well.

**Conclusion:** Current evidence suggest that there were significant differences in growth rate between each E. coli cell line. The most common broth (TB) used supported the growth rate the best and reached induction OD the fastest.

**Keywords:** protein expression, Escherichia coli, culture media, E. coli cell lines

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 72

### **Microtubule-associated tau in breast cancer**

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**Background:** Tau is microtubule associated protein that provides function of stability in cytoskeleton and shows high expression in breast cancer cells. Reports have demonstrated an abatement of tau is displayed in radiated cancer cells but it's still unclear how this loss will affect cellular function. In this study we will be delineating tau's role in breast cancer cells with the objective of analyzing tau expression, pre and post radiation using cancer cell line, MCF-7.

**Methods:** Tau expression was visualized by performing immunofluorescence on MCF-7 under a microscope.

**Results:** Interestingly the cells that have phospho tau (pTau) expression are also undergoing DNA damage repair and have nuclear localization with total tau and pTau. Additionally, total tau and DNA damage repair protein ( $\gamma$ H2AX) exhibits colocalization in the nucleus, a strong indication that both proteins are interacting.

**Conclusion:** Therefore this indicates that tau has a potential nuclear function in radiated breast cancer cells and DNA damage repair proteins like  $\gamma$ H2AX colocalizes with both total and ptau, further indicating tau's novel roles in cancer cells exposed to radiation. This finding could bring us one step closer into understanding tau's patterns and function in breast cancer cells.

**Keywords:** Tau protein, MCF-7, Breast cancer, Radiation

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 73

### **The Association Between Persistent Poverty and Melanoma Mortality in Texas: A Retrospective Study Using Texas Cancer Registry Data**

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**Background:** Patients in United States counties with persistent poverty (PP,  $\geq 20\%$  of residents in poverty since 1980) experience significantly higher cancer associated mortality than individuals living in non-PP counties. While markers of lower socioeconomic status have been correlated to higher melanoma mortality, the relationship of persistent poverty and melanoma mortality has not been explored.

**Methods:** We obtained data from 87,713 patients diagnosed with melanoma between 2000-2018 from the Texas Cancer Registry. We used 2021 US Economic Development Administration data to identify PP counties. Mixed effect models were used to assess incidence-based melanoma-specific mortality (IMM) rates by PP, age, sex and race/ethnicity.

**Results:** In 2021, 56 of 254 Texas counties met PP criteria. Of those diagnosed with melanoma, 5,431 patients in PP counties experienced higher IMM (17.14%) than those in non-PP counties (11.34%, adjusted odds ratio (AOR) = 1.10, 95% CI: 0.94-1.28). Compared to Non-Hispanic (NH) Whites, NH Blacks experienced the highest IMM at 25.67% (AOR = 2.97, 95% CI: 2.45-3.62), followed by Hispanics (19.71%, AOR = 1.98, 95% CI: 1.82-2.16). The disparity in IMM increased with age, with the 80 years + age group having the highest mortality AOR of 2.53 (95% CI: 2.16-2.96). When exploring sex, more men (13.43%) than women (9.09%) died from melanoma in PP counties (AOR = 1.48, 95% CI: 1.42-1.55).

**Conclusion:** Age, race/ethnicity, sex, and PP are associated with significant disparities in melanoma mortality outcomes within our patient cohort. To address these disparities and develop targeted melanoma secondary prevention interventions, we will continue to explore how place-based social determinants of health correlate with melanoma outcomes.

**Keywords:** Melanoma, Mortality, Texas, Persistent, Poverty

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 74

### **Using CRISPR for gene editing in *Caenorhabditis elegans***

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**Background:** CRISPR/Cas9 is a system discovered in *Escherichia coli* and now used as a tool in many different model organisms to edit specific genes. We used CRISPR in *C. elegans* to tag the gene *lin-53* with mRuby, a fluorescent protein.

**Methods:** We designed a crRNA to target *lin-53* as well as a repair template to facilitate homology directed repair. We built the repair template using PCR, purified it, and used it in our injection mix. We injected 25 worms with our repair template and a co-CRISPR to screen for edited animals.

**Results:** After 72 hours we found 220 potentially edited animals, put them on individual plates, then we used PCR to genotype them and find which potentially had the mRuby tag. Of these, 31 appeared to have the desired edit. We picked 8 of these to sequence because they had visible red fluorescence under the microscope.

**Conclusion:** We use CRISPR because it's an extremely efficient, straightforward way to edit a gene. The use of CRISPR/Cas9 allows us to target specific genes to edit using crRNAs as guide sequences, which allows us to insert a fluorescent tag on almost a protein of our choosing.

**Keywords:** CRISPR, *lin-53*, *C. elegans*, gene editing

Partnership for Careers in Cancer Science and Medicine



Abstract Number: 75

### **Exploring the Use of a Restoration Step to Detect Mosaic Chromosomal Alterations in Prostate Samples**

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**Background:** Prostate cancer is the most common cancer (besides skin cancer), and the second leading cause of death among American men. Genetic instability is a hallmark in tumor prevalence, resulting in mosaic chromosomal alterations (mCAs), including gains and losses. Although prostate cancers exhibit a lower mutation burden than other malignancies, mCAs are a relatively common mutation type within this cancer and tend to be prognostic. The purpose of the study was to assess the relationship between mCAs and cancer outcomes. It is useful to study cohorts with historical biospecimens and long-term follow-up. However, this means the samples will be stored for a long period in formalin-fixed and paraffin-embedded (FFPE), which over time further degrades DNA through cross-linking. Here we assess the impact on a cost-intensive restoration step in terms of mCA detection.

**Methods:** This pilot study consisted of an extensive analysis of prostate samples obtained through the Health Professionals Follow-up Study (HPFS). We studied 24 DNA samples from 10 participants, with varying concentrations of DNA. Each sample was processed two ways: with and without a restoration step to address damage from FFPE, resulting in 48 samples. We applied DNA SNP microarrays to these 48 samples and applied a mCA detection software to the data. The methodologies (non-restored vs. restored) were assessed based on: (1) genotype distribution and call rates, and (2) number of detected mCAs. A component of our comparisons involved the DNA quantities; some samples had less than the recommended 200ng. The restoration step itself further consumes DNA, resulting in some samples having as few as 9ng of DNA present.

**Results:** Our analysis found that the restoration step generally resulted in a higher genotype call rate. Consistent with this, we detected one third more mCAs with the restoration step.

**Conclusion:** Overall, the restoration step seems to be the more powerful method. We found the higher number of mCAs to be encouraging given the low false positive rate of the detection method. A drawback of the restored method is that it is three times more costly for the specific array used here, thus considerations may be made about power vs. sample size. This pilot informs a larger study with 1,000 patients with advanced prostate cancer and 1,000 patients without advanced cancer.

**Keywords:** chromosomal alterations, SNPs, archival samples, prostate cancer

Partnership for Careers in Cancer Science and Medicine

### **Cytotoxic potential of Mithramycin against DIPG cell lines**

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**Background:** Diffuse Intrinsic Pontine Glioma (DIPG) is a glial tumor of the pons with an extremely poor prognosis and limited treatment options due to its location in the brain stem. Current treatment involves radiation, but survival rates remain dismal. Thus, our goal is to identify novel oncogenic targets in order to develop effective targeted therapeutics. To identify novel targets, we performed integrated analyses of RNAseq and ATACseq of three different DIPG cell lines. This led to the identification of RE1 Silencing Transcription Factor (REST) and SET Domain Bifurcated Histone Lysine Methyltransferase (SETDB1) regulated genes as highly altered in human DIPG cell lines. REST is a repressor of neuronal genes, and SETDB1 is involved in the silencing of euchromatic genes, in contrast to the SuVAR family of proteins, which control heterochromatin formation. Expression of REST and SETDB1 is elevated in human DIPG tumors and cell lines. Co-immunoprecipitation demonstrated a direct physical interaction between REST and SETDB1 in a subset of DIPG cells. REST has been previously shown to be important for DIPG tumor growth. More recently, knockdown studies confirmed that SETDB1 is required for DIPG growth as well. REST and SETDB1 bind the upstream regulatory sequences of a target gene called CAV1, which is involved in several cellular processes including protein trafficking. Thus, we hypothesize that the REST-SETDB1 complex activity can be therapeutically targeted in DIPG cells. Mithramycin is an anti-cancer antibiotic produced by the bacterium *Streptomyces plicatus*. It binds GC-rich regions on DNA and thus, interferes with the binding of proteins that recognize and bind GC-consensus sites on DNA. Mithramycin is known to block the binding of Specificity Protein 1 (SP1) to DNA, downregulate the expression of the X-linked inhibitor of apoptosis protein (XIAP), and induce cancer cell death. SP1 is also required for the upregulation of SETDB1, and could potentially decrease its expression in DIPG cells. Here, we provide evidence that Mithramycin potently decreases the growth of DIPG cells in vitro.

**Methods:** We used DIPG cell lines (IV, IV-REST, VII, VII-REST, XIII, XIII-REST) and performed MTT cytotoxicity assay and cell cycle assay upon treatment with Mithramycin.

**Results:** REST and SETDB1 are overexpressed in DIPG tumors and contribute to proliferative potential. Interaction of REST and SETDB1 may be indicative of cooperative oncogenic behavior in DIPGs. Mithramycin treatment results in an increase in sub-G1 cell population in the three DIPG cells under study. Interestingly, REST elevation caused an increase in the sub-G1 population as well as cells arrested in S-G2/M phases of the cell cycle.

**Conclusion:** Mithramycin potently blocks the growth of DIPG cell lines in vitro. The underlying mechanisms remain to be evaluated. The role of REST in modulating DIPG response to Mithramycin also remains to be understood.

**Keywords:** DIPG, REST, SETDB1, Mithramycin, MTT

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 77

### **The Role of Radiation Therapy on Postmastectomy Breast Reconstruction Patients**

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Melissa Mitchell, Radiation Oncology, University of Texas MD Anderson Cancer Center

**Background:** Postmastectomy radiation therapy (PMRT) following breast reconstruction is among the leading choices of treatment in patients with breast cancer. However, various reconstruction complications have been linked to this approach. While some research has sought to identify covariates, which influence the rate of complications, there lies a gap in the literature. Our research assesses the impact of radiation therapy (RT) on complications for patients undergoing implant-based reconstruction following mastectomy.

**Methods:** A retrospective cohort study was conducted in patients who underwent mastectomy followed by breast reconstruction from October 2016 to December 2019. We reviewed charts from 548 patients undergoing mastectomy in 765 breasts, planned for implant-based breast reconstruction using tissue expanders (TE). Identified breasts were separated into four groups: mastectomy without RT (n=566), mastectomy with TE removed before RT (n=20), mastectomy with TE in place during RT (n=156), and TE implanted in a prior radiated breast (n=23). Categorical variables were compared using the Chi-squared test. P-values less than 0.05 were considered statistically significant.

**Results:** The most common complication type was infection (34%), followed by seroma (22%), wound dehiscence (13%), necrosis (12%), implant exposure (9%), hematoma (4%), deflation/rupture (4%), and capsular contracture (2%). The complication rate was highest among breasts with TE removed prior to RT (95%), followed by a complication rate of 45.8% in breasts with prior RT. 38.1% of breasts with TE in place during RT developed complications. Those breasts without RT developed the least number of complications (P= .000). In breasts that received RT, half of complications occurred over 10 months following surgery. Contrastingly, among those that did not receive RT, majority (55.2%) of complications took place within one month.

**Conclusion:** Postmastectomy breasts that did not receive RT developed the least number of reconstruction complications. Breasts that had undergone RT with TE in place had a higher risk for infection, seroma, and implant exposure (P < .05). Majority of the complications in breasts without RT took place within one month of the reconstructive procedure while most of the complications in breasts with RT took place months later.

**Keywords:** PMRT, implant-based reconstruction, tissue expander

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 78

### **Creating an Inexpensive Medical Linear Accelerator for Training and Educational Purposes**

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Laurence Court, Radiation Physics, University of Texas MD Anderson Cancer Center

**Background:** Medical Linear Accelerators (LINAC) are at the frontline of helping patients battle cancer through radiotherapy (RT) treatment. LINACs create high-energy X-rays which can kill cancer cells. The innovation of LINACs has helped save many patients' lives; however, this radiotherapy machine is extremely expensive—costing up to 4 million dollars. LINACs are constantly in use, making it difficult for trainees and patients to familiarize themselves with the RT machine. The development of a scale model of the LINAC would help facilitate patient and trainee education about the RT machine's functions and treatment expectations.

**Methods:** LINAC scale model that could be built using cardboard and other inexpensive materials, with movement controlled using UNO R3 Controller (Arduino) as the source powering the model. Arduino Software (IDE) was used to code Arduino.

**Results:** We designed the LINAC scale model to include four major parts, including the gantry, collimator, couch, and a platform. Two UNO R3 Controller Boards (Arduino) were used, one located in the gantry's stand and another in the platform. The gantry required rotation of 180 degrees clockwise and counterclockwise from the vertical. The collimator, attached to the gantry, required rotation of 180 degrees clockwise and counterclockwise from neutral. The 28BYJ-48 Stepper Motor (SM) was used to accomplish these movements since it required no tuning and was the most cost-effective. The couch had two distinct movements: up and down and 90 degrees from neutral. The SM was implemented in a scissor lift to produce the up and down movement of the couch and the Servo Motor SG90 (Servo) was implemented for the rotation of 90 degrees from neutral. To control the movement of each motor, joysticks were used for the SM and a potentiometer controlled the Servo. The 43.18cm x 20.32cm x 7.62cm platform was made to hold the couch and gantry in one place as well as the Servo needed for the couch.

**Conclusion:** We successfully designed and built a low-cost scale LINAC model that could be used for patient and staff education

**Keywords:** Medical Linear Accelerator, Scale Model, Training and Patient Education

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 79

### **Soluble E-Cadherin in regulation of ROS in Inflammatory Breast Cancer**

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**Background:** Breast Cancer is the most common cancer in women and is the second leading cause of death. Inflammatory Breast Cancer (IBC) is an aggressive variant of breast cancer with a strong propensity to spread to distant organs. Although only making about 1-4% of all breast cancer patients, it accounts for 8-10% of all breast cancer-related deaths. The mechanisms underlying the aggressive biology of IBC are unknown and no specific biomarker for IBC has been identified to date. An increase in the expression of E-cadherin, an epithelial adhesion molecule, has been noticed in tumors from IBC patients and cell lines. In preliminary work, soluble E-cadherin, an extracellular fragment of Full-length E-cadherin, was shown to promote tumor growth and survival of IBC cells. Also, Reactive Oxygen species (ROS) signaling is enriched in soluble E-cadherin-expressing IBC cells. The purpose of this study is to determine whether soluble E-cadherin promotes survival of IBC cells by regulating ROS.

**Methods:** The levels of ROS were compared between two groups, a control and Soluble E-cadherin overexpression SUM149 IBC cells. To achieve this, we performed culturing of cells, staining the cells with DCFDA and then quantifying by using a flow cytometer machine. We repeated the experiments twice.

**Results:** Hallmark pathway analysis showed that ROS signaling is enriched in soluble E-cadherin-expressing IBC cells. We found that the SUM149 IBC cells with soluble E-cadherin overexpression have significantly lower ROS levels compared to the control group ( $p < 0.05$ ).

**Conclusion:** We conclude that soluble E-cadherin reduces the levels of ROS in IBC cells. This suggests that soluble E-cadherin may promote the survival of IBC cells by inhibiting ROS levels, sparing the IBC cells from ROS-mediated cell death.

**Keywords:** E-Cadherin, ROS, IBC, Soluble E-Cadherin

Partnership for Careers in Cancer Science and Medicine

### **Establishing Optimized Culture Conditions for Human Intestinal Organoids**

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**Background:** Pancreatic Adenocarcinoma (PDAC) is among the deadliest forms of cancer with a 5-year survival rate of 11%. A contributing factor of poor prognoses is that PDAC tumors are highly resistant to radiotherapy. A high radiation dose is difficult to achieve because of the toxicity to nearby healthy tissues like the intestine. Intestinal organoids, or enteroids, can help study the physiology and diversity behind PDAC. Organoids are a form of 3D cell culture that provide a tractable method of studying cellular interactions which are more representative of in vivo conditions than 2D cell cultures because they replicate nutrition gradients and drug sensitivity like human organs.

**Methods:** Since they can be cultured by harvesting cells from adult tissues, embryonic, or induced pluripotent cells, genetic variation across donors is preserved. By adding the proper growth factors, the pluripotency of these cells can be preserved, and they can organize in 3D spheroid structures in a basement membrane like material. Among the growth factors necessary are three proteins important to preserving the stemness and proliferative nature of the cells: Noggin, Wnt 3A, and R-spondin. The lab utilizes a L-WRN cell line which secretes these three proteins into their cell media. The media is then collected, filter-sterilized, and supplemented with other growth factors necessary for enteroid growth.

**Results:** In order to determine that the cultured media from the L-WRN cells had sufficient levels of these growth factors, we measured their concentration via an ELISA assay. The cultured media was found to have sufficient levels of the three growth factors. These enteroids could be differentiated by omitting stem cell inducing factors such as Wnt3a and R-spondin. Such differentiated enteroids had increased villi cell density.

**Conclusion:** We found that the conditioned media from L-WRN cells provides sufficient levels of the growth factors to maintain enteroids without variations between batches. Enteroids ability to be cultured in a period of 5-7 days, while preserving the cell diversity and genetic alterations make them a good vector for studying GI toxicity to therapies. Because cancer cases are diverse, this form of sampling provides a safe way to test the impacts of therapies on a more personal and representative basis.

**Keywords:** Enteroids, L-WRN cells, Pancreatic Adenocarcinoma

Partnership for Careers in Cancer Science and Medicine

### **PDX USE IN CLINICAL TRIALS FOR TREATING COLORECTAL CANCER**

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**Background:** Colorectal Cancer is the third most commonly diagnosed cancer in men and women combined in the U.S. The second leading cause of cancer death in men and women combined in the U.S. As the number of younger colorectal patients rises, a colonoscopy is now recommended at 45 instead of 50.

**Methods:** Clinical trials are a way for researchers to test new drugs or combinations of drugs more effectively. A PDX model is tumor tissue taken from a patient and implanted into mice for research purposes, to test Cancer drugs and other types of treatment to see how well they work before they are given to the patient. Clinical Trial 2019-1016 is researching the best dose of LY3214996 WITH CETUXIAMB alone or with ABEMACIABLB E for patients with colorectal cancer that cannot be removed with surgery or has spread to other parts of the body. LY3214996/ AMEMACICILB may stop the growth of tumor cells by blocking some enzymes needed for cell growth. Cetuximab is a monoclonal antibody that may infer tumor cells' ability to grow and spread. Blood and tissue samples with predictive markers activity for immune effects for the treatments. Surgical implanted the tumor Subcutaneous into immunocompromised mice, in matched patients with cetuximab refractory metastatic colorectal cancer. The tumor was removed and expanded the tumor into more immunocompromised mouse. Molecular profiling can be done on the tumor with PCR or western blots. Afterward, the tumor is expanded into more mice to test the combination of mice with LY3214996 with CETUXIAMB alone or with ABEMACICLIB in two different sets of mice to test for biomarker response and resistance in LY3214996 with CETUXIAMB and LY3214996, CETUXIAMB, and abemaciclib.

**Results:** After testing on mice and if there is not much progression of the tumor and low toxicity, the trial can move into patients. Patients are divided into 2 arms, both starting with dose escalation. The 1st arm is Erk ½ inhibitor LY3214996 orally and cetuximab over 1-2 hrs. The 2nd arm is Erk ½ inhibitor LY3214996 and cetuximab as in arm a and ABEMACILIB PO twice daily.

**Conclusion:** The PDX model is an effective way of treating patients with colorectal cancer as it somewhat predicts the toxicity and its effectiveness. Protocol 2019-1016 is still ongoing therefore there are currently no results but its primary outcome measure is overall response and best response

**Keywords:** Colorectal Cancer, PDX Model, Clinical Trials, Cetuxiamb, Abemaciable

Partnership for Careers in Cancer Science and Medicine

### **MALT1 Inhibitor as a Therapeutic for Sézary Syndrome**

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**Background:** Therapeutic options for treating Sézary Syndrome (SS), an aggressive cutaneous T-cell lymphoma (CTCL), are currently limited. Thus, there is a critical need to develop enhanced treatment strategies. Genomic profiling of SS patients has revealed activating mutations in CARD11, a mediator of NF- $\kappa$ B activation through complex formation with BCL10 and MALT1. Selective inhibition of MALT1, a proteolytically active component of the CARD11-BCL10-MALT1 (CBM) complex, has proven efficacious in decreasing cell proliferation and inducing apoptosis in adult T-cell lymphoma and Diffuse Large B-Cell lymphoma cell lines overexpressing CARD11. Our study aims to demonstrate MALT1 inhibitors as a potential therapeutic for SS.

**Methods:** Immunoblotting was performed to assess expression levels of MALT1, BCL10, and/or CARD11 in four CTCL-derived cell lines (MJ, Myla, HH, and Hut78), CD4+ SS cells (n=5), and CD4+ T-cells from healthy donors (n=3). Three MALT1 inhibitors - Mepazine, Thioridazine and MI-2) were used to treat CTCL-derived cell lines at a series of concentrations (0.1, 0.5, 1.0, 5.0, and 10.0  $\mu$ M). RealTime-Glo MT Cell Viability Assay was used to assess the inhibitory effects by inhibitors on cell proliferation at 6 time points (3, 6, 18, 24, 48, 72 hours).

**Results:** We were able to confirm that MALT1 and BCL10 expression was upregulated in CD4+ SS cells compared to CD4+ T-cells from healthy donors. Of note, the levels of MALT1 and BCL10 proteins in CD4+ SS cells were parallel to absolute SS cell counts in patients. Three proteins (CARD11, BCL10, MALT1) in CBM complex were highly expressed in 4 CTCL-derived cell lines. Thioridazine and MI-2 had more inhibitory effects on cell proliferation than Mepazine. HH cells were more sensitive to inhibitors than MJ and Myla cells. Overall, the inhibitory effects of Thioridazine and MI-2 on CTCL-derived cell lines showed a time and dose-dependent manner.

**Conclusion:** Our results suggest that Thioridazine and MI-2 decrease cell proliferation in CTCL cell lines. More experiments are ongoing for dosages and mechanisms of action. Since thioridazine is well-tolerated and FDA-approved for treating anxiety disorders, this project has the potential to be directly and swiftly translated into the clinical setting.

**Keywords:** Sézary Syndrome, CARD11, MALT1, NF- $\kappa$ B

Partnership for Careers in Cancer Science and Medicine



Abstract Number: 83

### **Exploring the Effects of Oncolytic Viruses on the NK Cell Killing of Solid Tumors**

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**Background:** Natural killer (NK) cells are integral components of the innate immune system which recognize and lyse virally infected cells and tumor cells. However, NK cell therapy is currently mostly being used to treat liquid tumors. This is because solid tumors exhibit severe tumor hypoxia and maintain a suppressive tumor microenvironment (TME) through the secretion of NK sensitive-inhibitory cytokines, for example, TGF-. TGF- is an immunosuppressive cytokine released into the tumor microenvironment and generally functions to inhibit inflammatory immune cells including NK cells from killing a tumor. Additionally, these tumors do not express many of the activating ligands that NK cells use to immediately recognize and kill these malignant cells, allowing them to hide in the body. Ultimately, this presents challenges for NK cell therapy development for some of the most aggressive cancers in the body, including glioblastoma (GBM) and pancreatic ductal adenocarcinoma (PDAC). Oncolytic viruses are genetically-engineered viruses to specifically target and infect tumor cells rather than normal cells. This includes the 'oncolytic-Herpes Simplex Virus (oHSV)' and 'Delta-24-RGD (Adenovirus).' Due to NK cells' ability to recognize virally infected cells, we hypothesize that the infection of solid tumor cells with oncolytic viruses will increase NK anti-tumor lysis. In addition, the virus is a pathogen that can induce a local inflammation at the tumor site which can reverse the inhibitory nature of the TME to help NK infiltration and killing of the tumor.

**Methods:** In this study, we use xCelligence and Incucyte to monitor GBM and PDAC viability over time after the infection of the tumor cells with oHSV/delta24RGD and the addition of NK cells. Next, we explore the mechanism behind the virus-induced enhancement in NK killing through a preexposure assay. We use magnetic bead isolation to purify NK cells from coincubation with infected tumor cells and expose them to fresh tumors to observe the effects on NK cytotoxicity. Finally, we investigate the mechanism behind the enhanced cytotoxicity of dual knockout NK cells (knockout TGF- and glucocorticoid receptor), in combination with virally pretreated tumor cells. We pretreat NK cells with TGF- prior to tumor exposure and perform a TGF- titer assay in order to optimize this experiment.

**Results:** Overall, we find that oncolytic viruses enhance the NK cell killing response of solid tumors. Additionally, NK cell preexposure to infected tumors elicits a hyperactivation or memory-like behavior of these immune cells. Finally, we find that the dual knockout receptor of TGF- increases the probability of survival in vivo.

**Conclusion:** N/A

**Keywords:** NK Cell, Oncolytic virus, TGF-, Glioblastoma, Pancreatic Ductal Adenocarcinoma

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 84

### **Epigenetic Regulator, KDM4C, is Overexpressed in Pancreatic Cancer**

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**Background:** Pancreatic cancer is one of the deadliest types of cancer as it has no early signs and spreads rapidly to surrounding organs. Pancreatic cancer begins as a non-invasive ductal lesion and progresses into an invasive pancreatic ductal adenocarcinoma (PDAC) which accounts for 95% of pancreatic cancer cases. KDM4C is an epigenetic regulator that removes methyl groups from methylated lysines in heterochromatin which leads to an open chromatin structure that allows gene expression to occur. KDM4C is overexpressed in brain, lung, and breast cancers. However, KDM4C has not been previously studied in pancreatic cancer. Therefore, we are investigating the role that KDM4C expression plays in PDAC progression.

**Methods:** Normal and pancreatic cancer tissue samples were collected from mice and stained using immunohistochemistry. Using immunohistochemistry aid in comparing the presence of KDM4C in normal versus pancreatic cancer samples. A cell proliferation assay and colony formation assay were performed using PDAC cell lines and two different KDM4C knockout clones. Finally, normal and pancreatic cancer mice samples were collected and western blotted to assess the presence of KDM4C

**Results:** Immunohistochemistry staining showed that the pancreatic cancer tissue samples had higher levels of KDM4C than the normal pancreas tissue samples. The western blots showed that KDM4C was present in the pancreatic cancer cell lines but not in the knockout cell lines. The cell proliferation and colony formation assays showed that in the presence of KDM4C, cells reproduced at a higher rate than in the knockout cell lines.

**Conclusion:** KDM4C was found to be overexpressed in pancreatic cancer in mice and human samples. KDM4C also played a role in cell proliferation. As a result, the connection between histone demethylases and tumorigenesis is further strengthened. There is potential for histone demethylases to be used as therapeutic agents and in cancer therapies.

**Keywords:** pancreatic cancer, KDM4C, over expression, PDAC

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 85

### **Creation and Characterization of Patient Derived Xenograft Animal Model of Chromophobe Renal Cell Carcinoma**

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**Background:** Chromophobe renal cell carcinoma is a rare type of cancer that arises from the intercalated cells of the kidney's collecting duct and makes up 5% of all total RCCs. Due to the infrequent incidences, treatment usually comes from other types of RCC, such as the clear-cell subset. CHRCC is unique due to its association with aneuploidy and elevated mitochondrial pathways. While studies are still in very early stages, exploitation of this unique metabolic nature could lead to new treatments of CHRCC.

**Methods:** Chromophobe Renal Cell Carcinoma PDX Animal model: Resected CHRCC tumors were implanted subcutaneously into Fox Chase mice. Two tumors were each placed into 5 different mice. Once the tumors reached an approximate size of 1500 mm<sup>3</sup>, they were harvested. The tumors were collected for analysis and passaged onto the next group of mice.

**Results:** Short tandem repeat fingerprinting was used to compare the original CHRCC patient tissue to the PDX tissue. The results indicated below demonstrate very similar morphological and genetic composition of the original tissue and two passages.

**Conclusion:** A CHRCC PDX animal model was generated. As of June 2022, the animal model is on passage 4. STR fingerprinting was used to determine that the PDX tumors and original patient tumors were still morphologically and genetically similar after multiple passages. The creation of the animal model is a substantial development in the process of creating a unique treatment for CHRCC. While still in early stages, the animal model could potentially be used to test any new treatments and their safety on a continuous supply of tissue before moving on to human testing.

**Keywords:** Chromophobe Renal Cell Carcinoma, OXPPOS, Passage, Patient-Derived Xenograft

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 86

### **Glycosylated Collagen Interaction with Cells Through DDRs and Integrin**

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**Background:** Discoidin domain receptors (DDR1 and DDR2) and Integrin Beta receptors bind to collagen in the extracellular matrix (ECM) to function as pathways for cells to communicate and adhere to the surrounding cells and environment. In cancers when DDRs and Integrin beta are dysregulated or mutated, cell proliferation and migration increases. Collagen inside the ECM is post translationally modified by an enzyme called Galactosylhydroxylsyl Glucosyltransferase (GGT) which glycosylates Collagen. It has been found that there are higher levels of glycosylated collagen in cancer tissue compared to normal tissues. In this experiment, we focused on adhesion of DDRs and Integrin Beta to collagen when collagen was deglycosylated and glycosylated to determine if these receptors were collagen glycosylated dependent.

**Methods:** We created 344SQ cells containing knocked down DDR1, DDR2 and Integrin Beta receptors through viral transfection. We then used collagen 4 to conduct adhesion experiments. In our experiment, the control group was left untreated (without PGGHG), and the experimental group was treated with PGGHG which deglycosylated the collagen.

**Results:** To ensure our knock down efficiency in our cells worked, we ran a western blot and determined that the receptors in our cells were knocked down. From this experiment, we saw no significance in collagen 4 adhesion ability of the 344SQ cells when the cells were knocked down with DDR1, DDR2 or Integrin beta. We did see significance (\*\*) in adhesion with our control cells that contained our receptors.

**Conclusion:** Our experimental results show that the receptors we are studying interact with glycosylated collagen, however, it appears that our results dismiss the idea that collagen glycosylation does not affect the DDRs and integrin beta receptors binding ability. This ultimately opposes our hypothesis that these receptors are dependent on glycosylated collagen to adhere. Our goal now is to determine if any receptors in the 344SQ cell line are collagen glycosylated dependent for adhesion. We also want to research more about the DDRs and Integrin Beta receptors to better understand how they work and if they are dependent on other variables to adhere effectively.

**Keywords:** Glycosylated, Collagen, Receptors, Adhesion

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 87

### **Developing an IF panel to examine Cyclin and CDK interactions in a Pancreatic Adenocarcinoma Patient Derived Xenograph**

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**Background:** Cyclins coordinate cell division by binding to their associated cdk's and maintaining genomic integrity by tightly controlling these processes. In cancer, cellular proliferation becomes dysregulated, implicating both cyclins and cdk's. Complexes of therapeutic interest are Cyclin E1 (CCNE1) and CDK2, which complexes during late G1/S phase, and Cyclin A2 (CCNA2) and CDK1/2, which are essential for S-phase progression. Inhibition of specific complexes is thought to be a therapeutic tool to cancer patients due to their role in cell proliferation and regulating cell cycle checkpoints and response signals. To evaluate the co-expression of CDK1&2 and their Cyclin counterparts, a multiplex panel was developed and tested on a Pancreatic ductal adenocarcinoma (PDAC) model.

**Methods:** Antibody validation was performed by creating positive and negative controls using siRNA knockdown of the gene of interest. Briefly, HCT116 cells were seeded at optimal density and transfected with 50nM of siRNA targeting gene using DharmaFect reagents. Cyclin A2 protein was induced by treating cells with aphidicolin at 10uM for 16hrs, 30uM for 4 hours, and released for 4 hours. Cells were collected 72 hours later for knock down validation. Cytoblocks were generated and then used for antibody validation in multiple staining protocols such as IHC (immunohistochemistry), Opal IF, and Opal Multiplex panel. The optimized multiplex panel was used to stain a PDAC PDX model. Slides were imaged using Vectra Polaris and co-expression analyzed using Indica Halo software.

**Results:** Western blots showed successful CCNA2 knockdown. In HCT116 cells, optimized conditions and overexpression of treated cells were observed. Cytoblocks of these transfected and treated cells showed appropriate IHC staining patterns with NTC (wild-type) showing low basal expression, siCCNA2 KD lacking expression and treated cell showing overexpression. Multiplex panel of Cyclin CDKs was developed. The expression and co-expression of Cyclins A2, E1 and CDK1 &2 in a PDAC PDX model was analyzed. Analysis showed that CDK levels were high (45% CDK1 vs 60% CDK2) and more Cyclin E1 expression. Further subcellular analysis discovered CDK1 was mostly located in the cytoplasm along with Cyclin A2, while CDK2 and Cyclin E1 was mostly nuclear.

**Conclusion:** Targeting certain entities regulating the cell cycle could be a valuable therapeutic tool used in cancer cell growth prevention and research. Multiplex immunostaining can elucidate cdk complexes and could be utilized to understand mechanism of response as well as inform on biomarkers for CDK inhibitors.

**Keywords:** Cell cycle, Cyclin-CDK complexes, PDAC/PDX model, Multiplex IF Panel

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 88

### **Barriers to Cervical Cancer Screening for Hispanic/Latinx Women in the Harris Health Safety Net System**

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**Background:** Cervical cancer screening in the U.S. involves Pap smears and testing for Human Papillomavirus (HPV), yet Harris County is in the worst 25% of US counties for cervical cancer incidence. Previous studies have found that Latina women have the highest risk for cervical cancer incidence but are the least likely to seek screening (Gauss et al., 2013), so the PRESTIS (Prospective Evaluation of Self-Testing to Increase Screening) Trial sends HPV self-sampling kits to eligible and randomized Harris Health patients. This study investigates whether primary language, English or Spanish, and birthplace affect cervical cancer screening barriers for Latinx women in the Harris Health system and what solutions can be implemented to overcome these.

**Methods:** After PRESTIS mailed self-testing kits, 143 telephone surveys were conducted in English or Spanish to inquire about experiences with the kit and their healthcare in general. We assessed five common screening barriers: discomfort, embarrassment, uncomfortable with a male provider, difficulty getting appointments, and fear of finding out they have cancer. We utilized chi square statistical tests to evaluate differences between participant groups and constructed cross tabulations for analysis; SAS Version 9.4 was used for the analyses. We compared barriers to participant's primary language, whether they were born in the United States or elsewhere, and to birthplaces: Mexico or Central and South America.

**Results:** Among the 143 Hispanic women, 64.2% of Spanish speakers agreed that pap tests are embarrassing compared to 39.1% of English speakers ( $P = 0.03$ ). Likewise, 70.0% of Spanish speakers stated that they would feel uncomfortable with a male provider administering the pap test compared to 47.8% of English speakers ( $P = 0.05$ ). Birthplace did not have significant effects on their answers for any of the barriers.

**Conclusion:** A language barrier could have caused the significant differences in responses for embarrassment and discomfort with male providers, indicating why more Spanish speakers agreed with these two reasons than English speakers. Further understanding of the mechanisms causing differences in cervical cancer screening by primary language should be prioritized. Because birthplace did not affect participant's responses, Latinx women may experience similar barriers to pap testing; analogous steps can be taken to address these issues. Educational programs must be developed for Spanish medical translators to help ease patient apprehensions about screening.

**Keywords:** Cervical Cancer, Screening Barriers, Hispanic Women, HPV

Partnership for Careers in Cancer Science and Medicine

## Targeted Combination Therapy Improves Erdafitinib Efficacy in Urothelial Cancer

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**Background:** Fibroblast growth factor receptor (FGFR)-3 aberrations occur frequently in urothelial cancer. These aberrations lead to oncogenic signaling through the MAPK and PI3K pathways, but also confer sensitivity to FGFR inhibitors. Erdafitinib (Erda) is a pan-FGFR inhibitor that is only currently approved for targeted therapy in advanced urothelial cancer. The efficacy of erdafitinib is short-lived as patients develop resistance within six months of treatment emphasizing the need for effective combinatorial approaches to further enhance outcomes in this disease subtype. The overall goal of the project is to identify modifiers of erdafitinib sensitivity in FGFR3-mutated urothelial cancer and improve erdafitinib response rate using combinatorial approaches. Our previous genome-scale knockout CRISPR/Cas9 screen identified CCNC/CDK8, IGF-1R/MAPK, PTPN11/SHP2 inhibitors as synthetic lethality candidates with erdafitinib. The objective of this study is to validate synergism between erdafitinib and Sel120 (a CDK8 inhibitor) or SHP099 (a SHP2 inhibitor) in an erdafitinib-sensitive cancer cell line.

**Methods:** UC14 (FGFR3 S249C) cells were seeded in a 12-well plate at a density of 12000 cells/well. After 24h, the cells were treated with DMSO, Erda 1uM, Sel120 1uM, SHP099 10uM, Erda 1uM +Sel1201uM, and Erda1uM +SHP2i 10uM. Each treatment condition was duplicated. After 1 week, the cells were fixed with cold methanol and stained with propidium iodide supplemented with RNase A for capturing in an Incucyte machine. Growth inhibition by each treatment was calculated. Synergism for Erda + Sel120/SHP099 was measured by dividing the growth inhibition value of combination by that obtained for each single agent. A value < 0.7 was considered synergistic. Lysates from drug-treated UC14 cells at 2h, 8h and 24h were blotted for pAKT, total AKT, pERK, total ERK, and GAPDH expression. Also, RNA sequencing data previously obtained for Erda- and Sel120- treated UC14 cells were analyzed for gene enrichment using GSEA4.2.3 software. The molecular signature database selected was C6 collection: oncogenic signature gene set, and p<0.05 was set as the threshold for screening significantly enriched pathways.

**Results:** Growth inhibition by Erda+SHP099 / Sel120 > Erda >>> SHP2i > Sel120 > DMSO, and the co-inhibition of CCNC/CDK8 or SHP2 shows a synergistic effect with erdafitinib. GSEA also reveals that Erda (1uM) as a single agent does not significantly suppress FGFR signaling (p-values = 0.06 and 0.14, respectively, for Erda+Sel120 normalized by Sel120 and Erda alone). Sel120 does not synergize with Erda due to combined suppression of the MAPK pathway. Its addition to Erda did not further suppress phosphorylated ERK or AKT. When Erda+Sel120 was normalized by Erda, keratin filament genes were up-regulated (ES=0.80; nominal p-val=0.00; FDR q-val=0.07) while Hippo signaling was down-regulated (ES= -0.68; p-val=0.00; FDR q-val=0.33).

**Conclusion:** The study shows that Sel120 synergizes with erdafitinib possibly via hippo signaling pathway down-regulation. A future study that involves a drug assay for Erda/Sel120 following genetic knock-out of keratin filament genes and over-expression of hippo signaling genes in UC14 cells will provide valuable insights on the mechanism by which synergism occurs.

**Keywords:** Erdafitinib, Synergism, Urothelial cancer, Sel120

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 90

**APR-246 induces ferroptosis and overcomes cisplatin resistance in ovarian cancer.**

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**Background:** Ovarian cancer is the fifth deadliest cancer in women and is predicted to cause nearly 13,000 deaths in the United States in 2022. Ninety-six percent of high-grade serious ovarian cancers express mutations in p53 which is associated with more aggressive disease while 15-30% of patients develop resistance to platinating agents. APR-246, a thiol reactive compound, has been used in several clinical trials for solid tumor malignancies (NCT 04383938) due to its ability to restore mutant p53 function. Recently, it was shown that it also targets Thioredoxin-1 and GSH deficiency, thus inducing oxidative stress. Here we report that APR-246 targets ovarian cancer cell line A2780 for ferroptosis, an iron dependent cell death, independently of p53 status and can overcome cell resistance to cisplatin therapy.

**Methods:** We used isogenic A2780 cell lines (WT and p53 knockout) to test for the role of p53 as well as A2780 Parental and Cisplatin-resistant cell lines to test APR-246's ability to overcome cisplatin resistance. Cell viability was assessed using double negative Annexin-PI by flowcytometry. Assessment of cellular ROS was accomplished using CellROX Deep Red Reagent and lipid peroxidation was detected using C11-Bodipy by flowcytometry. GSH concentration was assessed using a 96-well plate luminometer. Cells were plated and attached overnight prior to drug incubation according to experimental setup and subsequent extraction with 0.25% trypsin.

**Results:** APR-246 induced apoptosis independently of the p53 status. APR-246 also induced GSH deficiency associated with increased cellular ROS and lipid peroxidation. The use of antioxidants (e.g., GSH/catalase) or anti-lipid peroxidation (Iiproxstatin/ferrostatin) rescued cell death, indicating a possible role for ferroptosis. Interestingly, APR-246 synergistically augmented cell death when combined with cisplatin (causing a 75% decrease in IC50) and overcame resistance to cisplatin in A2780 cisplatin-resistant cell line.

**Conclusion:** Ultimately, the GSH deficiency induced by APR-246 is associated with ferroptosis in the ovarian cancer cell line A2780 as evidenced by increased lipid peroxidation and oxidative stress, which is rescued by ferroptosis inhibitors and antioxidants, respectively. Catalase, specifically, can rescue ferroptosis by mitigating the oxidative stress necessary for lipid peroxidation which is induced by APR-246. The combined effect of APR-246 and cisplatin on cell death in the A2780 parental cell line is synergistic while overcoming cisplatin resistance in the resistant cell line.

**Keywords:** Ovarian cancer, APR-246, glutathione deficiency, ferroptosis, cisplatin resistance

Partnership for Careers in Cancer Science and Medicine



Abstract Number: 91

### **Role of Wilms' Tumor 1 in Sex Development**

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**Background:** During the development of male and female embryos, both individuals develop two pairs of epithelial ducts (the Wolffian and Müllerian ducts). The Wolffian duct differentiates into the male-specific sex organs, the epididymis, seminal vesicles, and vas deferens. The Müllerian duct differentiates into the female-specific sex organs, the uterus, oviduct, cervix, and upper portion of the vagina. In males, the Müllerian duct will be eliminated by the presence of anti-Müllerian hormone (AMH) which is produced in the testis, and the Wolffian duct will differentiate into the male reproductive tract. AMH is able to signal to its receptor, AMHR2, which is found in the Müllerian duct mesenchyme. Mutations in *Amhr2* can lead to persistent Müllerian duct syndrome in which males have both male and female reproductive tracts. Previous data shows that Wilms' Tumor 1 or WT1 is coexpressed with *Amhr2* in the MD mesenchyme prior to and during regression. Our goal is to answer the question "Is the expression of *Wt1* in the Müllerian duct mesenchyme necessary for the regression of the Müllerian duct?".

**Methods:** We use a tissue-specific CRE recombinase to interrupt *Wt1* signaling in the MD mesenchyme to prevent expression of *Amhr2* prior to regression of the Müllerian duct in male mice. If WT1 is a required activator of *Amhr2*, this should result in a male mouse with a retained uterus. Furthermore, we use histology, microscopy, and fluorescent imaging to analyze our data.

**Results:** Based on our histology data, when compared to the control uterus and vas deferens, the *Wt1* knockout mouse has both the vas deferens and uterus positioned next to each other. Fluorescent imaging reveals the presence of *Wt1* in the uterus of both the female and male reproductive tracts.

This data supports our hypothesis that the *Wt1* knockout mice will have both male and female reproductive tracts. Future directions include using qPCR to confirm normal AMH levels in the testes, and decreased *Amhr2* levels in the Müllerian duct mesenchyme after the knockout of *Wt1*.

**Keywords:** Sex development, anti-Müllerian hormone, sex differentiation

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 92

### **GeoMx digital spatial profiling (DSP) as an important tool to study immune landscape of tumors**

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**Background:** Immunoprofiling has become highly significant in order to understand in depth the immune microenvironment of tumors and their spatial relationships. NanoString GeoMx DSP is a new high-plex technology that allows the assessment of multiple proteins or RNA in a single slide, promoting the identification of targetable biomarkers in both tumor and microenvironment. We aim to describe the standard technical overview of the protein panel of this assay in normal tonsil tissue.

**Methods:** We used a 4µm FFPE section of a reactive tonsil to run a Human Immuno-Oncology Protein Panel (24 antibodies) in GeoMx DSP assay. In the first day, we perform the tissue and reagents preparation, followed by washing, staining, scanning, and region of interest (ROI) selection in the second day. Indexing oligos were released by the Sequential UV light exposure of each ROI were and quantified on NanoString's nCounter system. The data was exported and analyzed. Heat maps and box plots were used to visualize the different levels of protein expression.

**Results:** A total of 9 regions of interest were selected, passed by quality control, and analyzed. After the normalization using the signal background ratio, fifteen biomarkers showed median counts higher than one. Among them, we analyzed the different distribution in tonsil compartments of Pancytokeratin, CD3, CD4, CD20, CD45, CD68, Granzyme B, and Ki-67. The germinal center areas were characterized by the high expression of CD20 and Ki-67, while the interfollicular areas showed a high concentration of T-cell markers, such as CD3, CD4, and Granzyme B. The macrophage marker CD68 had a slightly higher concentration in germinal centers and there was no difference between the concentrations of the pan-immune marker CD45 between germinal center and interfollicular areas.

**Conclusion:** An adequate protein distribution pattern for typically expressed markers were seen in different compartments of a normal tonsil. GeoMx DSP is a robust and promising high-plex technology for the study of spatial protein immune landscape and can represent a great tool for translational cancer research.

**Keywords:** immunoprofiling; DSP GeoMx; protein; tumor microenvironment

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 93

### **Characterizing Novel Kras/p53 Derived NSCLC Cell Lines**

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**Background:** Lung Cancer metastasizes by way of epithelial to mesenchymal transition (EMT). Lung cancer can exhibit a range of epithelial and mesenchymal characteristics. Epithelial cells have polarity and tight cell junctions. Mesenchymal cells lack polarity and are more invasive. The lab has previously derived a panel of cell lines from the genetically engineered mouse KrasG12D/+; p53R172HΔG/(KPAΔG): 393P and 344SQ. These cell lines were thoroughly characterized, and it was found that 393P is relatively epithelial and 344SQ is relatively mesenchymal (Don Gibbons, 2009). More recently, the lab has derived a new panel of cell lines from KrasG12D/+; p53R172H/R172H(KPMUT) mice: 7064P, 7362P, and 7578P. This project aims to characterize the novel cell lines on a scale of epithelial to mesenchymal using the known 393P and 344SQ cell lines as standards.

**Methods:** Brightfield images taken with a microscope to show the morphology at 10x and 20x. qPCR of known epithelial and mesenchymal markers was performed, data was normalized, and relative expression plotted with significance being determined by Student's t-test in PrismGraphPad. Western blot of epithelial and mesenchymal markers was performed, and images were taken on the BioRad Chemidoc using chemiluminescence. Migration assay of the five cell lines was performed by first seeding cells in triplicate into transwell chambers with serum-free media, and the chambers were placed in wells with complete FBS media. The cells were allowed to migrate for 16 hours. After which, the cells were fixed and stained in crystal violet for 24 hours. Five fields were captured per chamber and cells quantified using ImageJ.

**Results:** Morphologically, 393P and 7578P form tight clusters, while 344SQ, 7064P, and 7362P do not. Moreover, 344SQ, 7064P, and 7362P exhibit protrusions indicative of a mesenchymal nature. At the transcription level the data was inconclusive for the markers looked at; the cell lines showed both over and under expression for epithelial and mesenchymal markers. Functionally, we see different results at the protein level, but cells still seem to have contradictory pathways. Looking at the migration images, all of the cell lines have the capability to mount a migration.

**Conclusion:** The data suggests that the novel cell lines are more mesenchymal than 393P and more epithelial than 344SQ. The novel cell lines still lay within the two extremes of the control.

**Keywords:** NSCLC, Kras, p53, EMT

Partnership for Careers in Cancer Science and Medicine

### **The two faces of CD73 in tumor-infiltrating lymphocytes expanded from liposarcoma**

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**Background:** Adoptive cell transfer using tumor-infiltrating lymphocytes (TIL) has shown clinical benefits in metastatic melanoma as well as other solid tumor types [1]. Liposarcoma is a soft tissue sarcoma with less than 40% TIL expansion success rate without selection methods and a 60% success rate with selection based on at least 300 events of CD3+ T cells detected in the fresh tumor sample using flow cytometry. Previous studies in renal cancer carcinoma indicated that presence of CD73 is correlated with poor patient prognosis and immunosuppression in the tumor microenvironment (TME) [3]. We hypothesized that higher expression of adenosine pathway-associated markers, such as CD73, would result in a paucity of immune infiltration including TIL, and therefore influence TIL expansion success.

**Methods:** TIL expansion was attempted using the 'TIL 3.0 MDACC method' from a total of 18 surgically resected liposarcoma cases [3]. Briefly, five 1-3 mm<sup>3</sup> pieces were plated in a G-rex with media containing OKT3 (anti-CD3), agonistic anti-41BB, and IL-2. Every 3-4 days, media with IL-2 was refreshed. After 21 days, the cells are harvested and counted. Expanding (E) was defined as 40 x 10<sup>6</sup> TIL expanded based upon thresholds needed for clinical REP and treatment. Phenotypic analyses of the TIL populations prior to expansion were performed using flow cytometry. Statistical analysis was performed in GraphPad Prism (two means t-test Mann-Whitney).

**Results:** We first observed no significant differences of total immune infiltrate or lymphocyte subtype (CD45+, CD3+, CD8+, and CD4+) populations by percentage between expanded (E) and non-expanded (NE) liposarcoma TIL. Interestingly, CD8+ cells showed significantly higher CD73 expression (p=0.0441) in the expanded TIL group. No other lymphocyte subtype showed this higher expression of CD73. Additionally, there was no significant difference in CD73 expression on tumoral cells (CD45-) across groups. Expanded TIL also had significantly higher Lag3 (p=0.0443) expression in CD4+ cells but not in CD8+ cells. All the other explored molecules, including PD-1 and 41BB, showed no significant differences.

**Conclusion:** Immune cell subtype populations by percent do not affect TIL expansion. Conversely to previous studies, we demonstrated that expression of CD73 in the TME or on tumoral cells (CD45-) may not affect the expansion of TIL in liposarcoma and potentially promotes CD8+ T cell proliferation

indicating that CD73 is not completely an immunosuppressive molecule. Lastly, higher Lag3 in CD4+ in the expanded samples indicates that Lag3 is not behaving as a marker of T cell exhaustion as seen in previous studies [4]

Keywords: Tumor Infiltrating Lymphocytes, Immunology, Liposarcoma, CD73, Cell Proliferation

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 96

**Evaluation of newly generated LRP1 antibodies in different cell types: THP1 cancer cell line, human and mouse immune cells**

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**Background:** Increased knowledge surrounding the biology of T-cell activation and proliferation has enabled immunotherapy to combat various deadly diseases, such as cancer. Revealing the key molecules that play vital roles in T-cell proliferation is imperative for the development of new anti-cancer treatments and therapeutics. Dr. Mollrem's lab is focusing on ubiquitously expressed molecule - Low Density Lipoprotein-related protein 1 (LRP1), that has been only recently identified as a novel modulator of T-cell proliferation. In the lab's previous findings, LRP1 has been shown to play a direct role in T-cell proliferation as neutrophil mediated inhibition of PBMCs (human peripheral blood mononuclear cells) was partially rescued via commercially available anti human LRP1 antibody. To further study the function of LRP1 in T-cell biology, the lab created a knockout LRP1 T-cell mouse model, where LRP1 is deleted specifically in T-cells only. To validate this model, as well as study the LRP1's role in mouse T-cells in wild-type mice a reliable LRP1 antibody is required. However, there are zero commercially available LRP1-targeting antibodies that bind well to both human and mouse. There is a critical need in producing of an effective blocking antibody to study the potential functions of LRP1 in T-cell biology both in vitro and in vivo.

**Methods:** Our lab generated six different antibody clones against human LRP1. The goal of the project is to test each clone's ability to bind to both mouse and human immune cells utilizing Flow Cytometry method.

**Results:** Preliminary data shows there is significant binding in human monocytes compared with T-cells. On the other hand, flow cytometry revealed strong binding in mouse T-cells, monocytes, and neutrophils. Specifically, clones 1F7-1, 2A7-1, and 19D4-1 showed consistent binding in THP1 human monocytic cancer cell line, human healthy donor -derived monocytes, and mouse splenocytes.

**Conclusion:** An effective blocking antibody is needed to study the potential functions of LRP1 in T-cell biology in vitro and in vivo. In this experiment, three newly generated LRP1 antibodies showed significant binding in both human and mouse immune cells. The next steps of this project include determining which antibody has the strongest binding affinity to mouse and human LRP1, directly conjugate the antibody with fluorochrome to exclude using a secondary antibody, activate human T-cells to increase LRP1 surface expression and examine binding, and to validate LRP1 KO mouse T-cells model (negative control) with the chosen LRP1 antibody clones.

**Keywords:** Cancer Immunology, LRP1, Antibody, Flow Cytometry

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 97

**p0071-catenin may modulate dendrite morphology in a phospho-dependent manner**

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**Background:** Dendrite morphology is crucial for healthy neuron function, and abnormal morphology is associated with many diseases. Delta-catenin modulates dendrite morphology in a phospho-dependent manner. Phosphorylated  $\delta$ -catenin binds to Pdlim5, promoting dendritic branching, while unphosphorylated  $\delta$ -catenin binds to Magi1, promoting dendritic elongation. p0071-catenin is in the same subfamily as  $\delta$ -catenin, and they share a similar PDZ-binding motif to bind Magi1 and Pdlim5. Thus, we hypothesize that p0071-catenin will bind Magi1 and Pdlim5 in a phosphor-dependent manner to modulate dendrite morphology like  $\delta$ -catenin.

**Methods:** The ability of p0071-catenin to bind Magi1 and Pdlim5 was assessed via co-immunoprecipitation experiments in HEK 293 cells, then imaged via western blot. Morphological effects of p0071-catenin phosphorylation were assessed by transfecting phospho-mimetic and phospho-null mutants into primary rat hippocampal neurons. Dendrite length and branching were quantified dendrite morphology via Imaris.

**Results:** p0071-catenin was found to bind Magi1 and Pdlim5 via co-immunoprecipitation. Overexpressing phospho-mimetic and phospho-null p0071-catenin in neurons increased dendritic length, and overexpressing phospho-mimetic p0071-catenin in neurons increased dendritic branching.

**Conclusion:** While changing the phosphorylation state of p0071-catenin affects dendritic branching as expected, phosphorylated p0071-catenin was not expected to increase dendritic length. This could be caused by other potential binding partners interacting with p0071-catenin while phosphorylated, which would differentiate it from delta-catenin. Future studies will include co-immunoprecipitations and relocalization assays to determine if p0071-catenin binds Magi1 and Pdlim5 in a phospho-dependent manner.

**Keywords:** neurons, dendrite morphology, catenin

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 98

**Pancreatic Cancer: Looking at the Immunosuppressive Nature of PC (Pancreatic Cancer) in Relation to CD-8+ T Cell Expression and PD-L1 Expression Levels in Murine Tissue Models**

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**Background:** Pancreatic cancer has rapidly become one of the deadliest cancers worldwide. With overall survival rates and disease-free survival rates improving only minimally over the past 40 years, researchers have turned to cancer immunotherapy for answers. One of the key defining features of pancreatic cancer is its tumor microenvironment (TME) which is notorious for having a prominent desmoplastic microenvironment exceedingly high in T cells. When analyzing immunotherapies developed against pancreatic cancer a key factor that persistently limits therapeutic success is the lack of T cells in tumor cell regions, a profile termed “immune-excluded”

**Methods:** In this study I analyze the link between T cell expression in the TME on PC (pancreatic cancer) positive (Kras;ERT2) and negative (ERT2) mice models via IHC (immunohistochemistry) staining of the CD-8 surface marker.

**Results:** Findings from this study indicate through IHC that there is a noticeable difference in CD-8 counts in PC positive and PC negative murine models. PC positive models reported to have an average of  $21.190 \pm 5.367$  CD-8 stains and PC negative models having an average of  $8.184 \pm 4.602$  CD-8 stains at a 90% confidence interval and an average of  $21.190 \pm 6.367$  CD-8 stains and  $8.184 \pm 5.483$  CD-8 stains at a 95% confidence interval. Despite higher levels of CD-8 in the cancerous mice models, tumor cell-inherent resistance mechanisms in cancer positive models impeded proper T cell infiltration and therefore lead to a subpar immune response leading to the advancement of the cancer.

**Conclusion:** After having compared CD-8 expression in PC positive and negative models, I looked further into another study by the National Cheng Kung University in Taiwan comparing CD-8 stains and PD-L1 levels to further understand the role of CD-8 expression in relation to PD-L1 with regards to OS (overall survival) and DFS (disease free survival). The National Cheng Kung University in Taiwan established that low CD-8 cell infiltration and high PD-L1 expression significantly stratified patient survival. Applied to the case of pancreatic cancer in such a dense desmoplastic TME, T cell expression does not indicate that the body is lacking a proper immune response but that factors in the TME prevent proper infiltration of immune cells into tumor growth regions.

**Keywords:** Pancreatic Cancer, CD-8 Expression, PD-L1 Expression, Tumor Microenvironment, Cancer Immunotherapies

Partnership for Careers in Cancer Science and Medicine



Abstract Number: 99

## **Investigating the Relationship between CBP and CARM1 Within CBP Knock Out RL Human Lymphoma Cells**

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**Background:** Human lymphoma cells have an acute mutation in the CBP protein and the CARM1 gene affects lymphoma cells as well. CARM stands for Coactivator Arginine Methyltransferase, which means CARM1 increases gene expression through methylation. It has been noted from previous studies that the lowered gene expression of CARM1 shows a decreased methylation of lymphoma cells, which also slows their growth. CARM1 can methylate CBP, leading to the growth of Non-Hodgkin Lymphoma cells. There have been few studies behind the relationship between CARM1 and CBP, but not in depth.

**Methods:** CBP and CARM1's relationship is being investigated within this experiment. Given two forms of cells, RL WT and CARM1 RL KO cells. These cells are being created through the vector lentiCRISPRv2. First the plasmid DNA is mixed with the DNA of E. Coli, this is done to ensure the growth of the vector as E. Coli replicate quickly. After a colony has formed, it will be inoculated and grown. Four colonies are separated, and four different oligos (Guide RNA's) are created through the addgene software. After the CARM1 guide RNA is created the plasmid DNA is purified through an agarose gel extraction, removing the filler. The oligo is placed inside the plasmid, using T4 PNK and Ligation Buffer. The oligo is then annealed inside the plasmid through a heat shock and PCR. The vector is then placed into COS-1 cells. COS-1 cells are monkey kidney cells with the specific SV40 antigen. This antigen is crucial for growing lentiviral particles, as it binds to the receptor which then releases viral particles into the media. The lentivirus can then be harvested from the media. The virus is incubated with puromycin along two types of cells, RL CBP KO cells and RL WT cells. This vector gives the option of creating a gRNA that will target the exact sequence of CARM1 and lower its gene expression in both genes. The second version of cells is RL CBP KO cells, meaning they have no expression of the protein CBP. The lentiCRISPRv2 mechanism is very useful, as it can delete genes/proteins that are being investigated.

**Results:** The oligo successfully placed inside the plasmid DNA and the RL cells and have DNA sequencing of the CARM1 gene and gRNA scaffold being placed correctly. The RL human lymphoma cells have finished the puromycin selection, with the remaining cells containing the puromycin resistance gene.

**Conclusion:** The cells are soon to be tested using the T7E1 Assay and single cell colony paired with western blot. The T7E1 assay is important, as it will find the strands of DNA that did not perfectly match and will cleave the strands. It will also amplify these specific strands using PCR for further observation. The single cell colony is imperative, as it will have four colonies from each oligo from a single cell. The cells will be examined through western blot to test for gene expression of CBP and CARM1.

**Keywords:** oligo, CBP, CARM1, KO, methylation

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 100

## **Investigating the Effectiveness of Nanopore Technology and Hi-C Analysis in Detecting Structural Variations in Human Soft-Tissue Sarcoma Samples**

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**Background:** Structural variations (SVs) are the most disruptive mutation type in human tumors, affecting the largest fraction of the cancer genomes. Currently, detecting SVs in cancer genomes is challenging due to technical limitations and complexity of SVs. In this study, we evaluated two cutting-edge genomic techniques for SV detection in human soft-tissue sarcoma samples. Our first method is Oxford Nanopore technology which relies on a single strand of DNA to pass through a small protein channel (a nanopore) that is embedded in an electrically resistant membrane. As the strand of DNA passes through the pore, each base causes a characteristic current disruption which is then decoded using basecalling algorithms to determine the DNA sequence. Our second method to examine genomic rearrangements is Hi-C, a chromosome conformation capture method. Hi-C involves cross-linking DNA within cells to create a snapshot of which genomic regions physically interact with each other. Hi-C has been identified as an intuitive method for recognizing structural variations due to the large insert size and the detection of spatial proximity near breakpoints.

**Methods:** To investigate structural variation calls identified by Hi-C (using HiCBreakFinder algorithm) and Nanopore (using Sniffles algorithm) in human tumor samples, we used a data visualization tool, Juicebox. We looked at Hi-C contact maps for osteosarcoma and 2 different liposarcoma tumor samples. We analyzed the efficiency of Hi-C and Nanopore and their ability to identify structural variations in cancer cells by comparing the SV call annotations to the Hi-C contact maps. We noted patterns observed about where Hi-C and Nanopore would identify structural variations and conclude their efficiency by estimating the amount of calls in a locus compared to the rearrangements seen in the Hi-C contact maps.

**Results:** In general, Nanopore was proved to be better at identifying SVs inside TADs and in interchromosomal areas. Interchromosomal interactions are an indication of a rearrangement and a problem present in the chromatin organization of that cell. Hi-C did better at identifying SVs in intrachromosomal areas compared to Nanopore. Neither technologies were able to identify all of the genomic rearrangements.

**Conclusion:** Our study revealed that detecting SVs with a single method might have certain limitations. Therefore, we conclude that a combination of orthogonal methods is needed for precise SV detections in human tumor samples.

**Keywords:** Hi-C, Nanopore sequencing, Structural variations

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 101

### **Identifying Differences in Spatial Transcriptomics Between Subtypes of Pancreatic Ductal Adenocarcinoma**

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**Background:** Pancreatic ductal adenocarcinoma (PDAC) is a lethal cancer, ranking fourth in cancer-related deaths in the US. Tumors are frequently heterogeneous and have differing clinical outcomes, indicating further characterization of tumors into distinct biological subtypes would enable the development of more personalized and effective therapies. The delta method of CT image analysis is used to visually categorize tumors as high delta and low delta before treatment. High delta tumors are more conspicuous on CT, and patients with high delta tumors have earlier distant metastasis and shorter overall survival compared with patients with tumors classified as low delta, which are less conspicuous on CT. PDAC transcriptomic classification is used to categorize tumors as classical, which are frequently resectable, and basal-like, associated with poorer clinical outcome. This project aimed to find the spatial transcriptomic (ST) differences between high and low delta tumors.

We identified 20 PDAC tumor samples, ten classified as high delta and ten as low delta. Each sample was sectioned and placed on a Visium slide. The slides were hematoxylin- and eosin-stained and viewed on a light microscope before undergoing hybridization, ligation, and barcoding using the 10X ST protocol. The library construction of each sample was prepared, then sequenced and analyzed to investigate genetic differences.

**Results:** Preliminary results using one high delta and one low delta found differences in the types of genes expressed and the average expression of basal-like and classical gene markers. Ten genetic clusters were identified in both the high and low delta samples. Three of these clusters were found in both samples and the remaining seven were predominantly seen in either one of the samples. No cluster assessed contained predominantly the cancer areas. Analysis of these clusters indicated correlation to its respective morphology and cell composition

**Conclusion:** The preliminary data indicate differences in the transcriptional profiles of high and low delta tumors. Data analyses are ongoing and will be validated.

**Keywords:** pancreatic cancer, spatial transcriptomics, computer tomography, transcriptional profile

Partnership for Careers in Cancer Science and Medicine

Abstract Number: 102

### **Effects of Education, Income, Gender, and Age on Intention to Get the COVID-19 Vaccine**

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**Background:** Blacks are more likely to be adversely affected by COVID-19.<sup>1</sup> Studies have shown that those with higher education and income and those that are older are more accepting of the COVID-19 vaccine.<sup>2,3</sup> This study looks at the association between education, income, gender, and age with intention to get vaccinated against COVID-19 among Black adults. The hypothesis is that Blacks with higher education, income, and age will report greater intention to get vaccinated against COVID-19 than Blacks with lower education, income, and age.

**Methods:** This study used data from the CEAL study, which stands for Community Engagement Alliance Against COVID-19 Disparities. CEAL included a web-based pre- and post-survey to explore COVID-19 vaccine hesitancy among Blacks and Hispanics in the local community.<sup>4</sup> The pre-survey included self-reported demographics.<sup>4</sup> Subjects were recruited from the community with the help of an existing community advisory board.<sup>4</sup> The subjects in this study were Black, aged 18 or older, and did not receive any COVID-19 vaccine. There were 1064 subjects in this study.<sup>4</sup> The primary outcome is intention to receive a COVID-19 vaccine within the next 30 days.<sup>4</sup> Frequencies and descriptive statistics were used to describe participant characteristics. Unadjusted models were performed for each predictor to assess their relationship with the outcome in univariate logistic regression analyses. Finally, multiple logistic regression was used to include all significant predictors in the model.

**Results:** Those with a bachelor's degree or higher were more than three times as likely to report intention to get vaccinated in the next 30 days than those with less than a bachelor's degree. Those who were at least 30 years old were two times more likely to say they intended to get vaccinated in the next 30 days than those who were younger than 30. Lastly, those who made at least \$50,000 annually were one and a half times more likely to have intentions to get vaccinated in the next 30 days than those who made less than that. Gender was not significantly associated with intention to get vaccinated in the next 30 days.

**Conclusion:** Blacks with higher age, income, and education display greater intention to get vaccinated against COVID-19 in the next 30 days than those of younger age and with lower income and education. To continue to make progress towards herd immunity against COVID-19, it is important that public health officials, healthcare workers, and policy makers remain aware of this disparity amongst Blacks.

**Keywords:** COVID-19, vaccine, education, age

Partnership for Careers in Cancer Science and Medicine

### **Understanding the effect of stress hormones on ovarian cancer cells**

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**Background:** Ovarian cancer is one of the leading cancers in women and the fifth highest cause of cancer related death in women. Understanding the underlined mechanisms of poor prognosis in ovarian cancer patients will directly aid in developing better treatments. Recently, it has been found that ovarian cancer patients with poor prognosis have shown elevated stress hormones. So, in this summer, I worked on to understand the metabolic changes occur in ovarian cancer cells when they are treated with stress inducing hormones employing NMR spectroscopy-based metabolomics.

**Methods:** HeyA8 is a high grade ovarian serous adenocarcinoma human cell lines and used in this study. Metabolites were extracted from these cancer cells using a 2:1 methanol-water solvent. The mixture of cancer cells and ceramic beads were subjected to three cycles of mechanical homogenization and freeze- thawing process to extract metabolites. This is followed by centrifugation, rotary evaporation, and lyophilization to remove the solvent. The samples are prepared for nuclear magnetic resonance (NMR) spectroscopy by dissolving the sample in 2 H<sub>2</sub>O containing the reference compound 4,4-dimethyl- 4-silapentane-1-sulfonic acid-d<sub>6</sub> (DSS). The data was acquired on a Bruker NMR spectrometer operating 500 MHz <sup>1</sup>H resonance equipped with a cryogenically cooled triple resonance (<sup>1</sup>H, <sup>13</sup>C, <sup>15</sup>N) prodigy BBO probe. Identification of metabolite peaks was done through Chenomx and the Human

Metabolomic Database (HMDB); finally, the peaks were integrated in Topspin and normalized to the reference compound (DSS). All <sup>1</sup>H-NMR spectra were normalized to the cell count before analysis.

**Results:** The significant difference in metabolites glutamate, guanosine, and uridine was observed between control and cortisol treated ovarian cancer cells.

**Conclusion:** NMR spectroscopy-based metabolomics was able to establish the difference in metabolism of control and cortisol treated ovarian cancer cells. Initial results showed difference in glutamate, guanosine, and uridine levels in cortisol treated cells compared to controls. However, the obtained results need to be validated with analysis of a greater number of samples.

**Keywords:** NMR, metabolites, ovarian

Partnership for Careers in Cancer Science and Medicine

### **Project Self: Addressing Barriers to Recruiting Hispanic/Latinx Women in Houston Area Housing Site**

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**Background:** The objective of this Project Self is to examine the feasibility of conducting an education intervention trial regarding cervical cancer screening among women of Hispanic and African American origin living in public housing units in Houston, Texas. Potential participants recruited from Irvinton and Fulton Village, with Fulton Village having more Hispanic/Latinx resident population. Dr. Shastri and the Project Self research team worked with the Community Scientist Program to help improve Project Self's study design and recruitment materials. It is difficult to attract enough participants for research projects in general, recruiting non-white Hispanics to participate can be especially tough. Potential Hispanic/Latinx participants might distrust the research process; fear discrimination, loss of confidentiality, or other harms; or lack information about the research process and its value—and as a result they may be reluctant or completely unwilling to participate.

#### **Methods:**

- Literature Review on recruiting Hispanic/Latinx Women on community-based participatory research studies/clinical trials.
- In depth Interview with the Houston Housing Authority (HHA) Coordinator to learn more about Fulton Village resident engagement.
- Self Observations on how Project Self study staff addresses barriers to recruiting Hispanic/ Latinx women.

#### **Results:**

- Based on our recruitment experiences, we have seen both a high and low number of attendance at the participating housing sites.
- Out of the 34 interested forms completed, 7 forms were from Hispanic/Latinx potential participants; Fulton Village residents.
- HHA Interview
- “We must come up with creative ways to engage residents and have them attend events at the community center... In the past, we’ve had many events at the Community Center where very few residents attended...”
- “Compared to Irvinton Village, Fulton Village has more Hispanic/Latinx residents whose preferred language is Spanish. Recruitment materials need to be in Spanish...”

#### **Conclusion:**

There are several viable strategies for addressing barriers to recruiting Hispanic/Latinx populations:

- Collaborate, build and maintain relationships with the targeted community and community members.
- Suggestions from research staff: have study staff that can relate to your targeted population, based off race/ethnicity and preferred language (Bilingual; Spanish)
- Literature Review

- “People from non-White racial groups and other underserved populations, including Latinos, are frequently reluctant to participate in research...”
- A Personal Touch: The Most Important Strategy for Recruiting Latino Research Participants
- “Developing relationships with recruits expressed shared Hispanic cultural values of personalismo (preference for warm relationships that convey care and acceptance of the patient and [their] circumstances)...”

A Personal Touch: The Most Important Strategy for Recruiting Latino Research Participants

Keywords: Recruitment, Hispanic/Latinx, Women

Partnership for Careers in Cancer Science and Medicine

### **Effects of ZIC1 Silencing in Malignant Peripheral Nerve Sheath Tumors**

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**Background:** Cancer, broadly defined as the uncontrolled and unregulated growth of abnormal cells in the body, is the second leading cause of death in America. Within the field of cancer, sarcomas are a cancer classification that derive from cells within connective tissue. The malignant peripheral nerve sheath tumor (MPNST) is a specific type of sarcoma pathology thought to arise from the Schwann Cells of the peripheral nervous system. Currently, there are no effective therapeutic targets for MPNSTs, as they are notorious for their resistance to chemo and radiotherapeutic strategies. A novel field of research has positively correlated the upregulation of ZIC1 with polycomb repressive complex (PRC2) mutation status in MPNSTs. Interestingly, the upregulation of ZIC1 in these cell lines is thought to contribute to a reversion of MPNSTs to a neural crest (NC) phenotype, an early stage in CNS (Central Nervous System) neurogenesis where ZIC1 is transcriptionally active. Given the observed phenotypic and epigenomic differences in PRC2 mutated MPNST cell lines, and the prevalence of PRC2 mutation in MPNSTs (70-80%), our lab has identified ZIC1 as an experimental therapeutic target in this sarcoma subtype

**Methods:** We have utilized the CRISPR-dCas9KRAB system to target identified ZIC1 enhancers to obtain knockdown of gene expression. However, western blotting of enhancer silenced cell lines indicated no change of ZIC1 expression. Clonogenicity plating showed no difference of clonal formation in enhancer silenced cell lines, which aligns with the results of our western blots. To quantify any potential difference in proliferation within our cell lines, we utilized an MTS assay, which confirmed that there was no significant difference in proliferative rates between our lines.

**Results:** Currently, our project suggests that the transcriptional accessibility of currently identified ZIC1 enhancers may not be essential for basal levels of ZIC1 expression, and as such, an ineffective target for ZIC1 silencing.

**Conclusion:** Future studies should identify and investigate alternative enhancers of interest in relation to ZIC1 expression. ZIC1 knockout may be utilized to observe the qualitative effects of this gene in PRC2 mutated MPNST cell lines. Alternatively, it may prove prudent to investigate alternative genes associated with the NC phenotype, such as WNT3, FZD3, BMP7, and SOX9, which are also aberrantly expressed in PRC2 mutant MPNST tumors.

**Keywords:** silencing, MPNST, ZIC1, CRISPR-dcas9KRAB, therapeutic target

Partnership for Careers in Cancer Science and Medicine





## Radiation Oncology Medical Student Summer Research Program

Abstract Number: 103

### **A preliminary study of potential variations in toxicities from RT in early stage breast Cancer patients treated pre and post-COVID with attention to ultra-hypofractionation**

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Melissa Mitchell MD, Radiation Oncology, University of Texas MD Anderson Cancer Center

**Background:** During the weeks and months after COVID, when healthcare resources were scarce, then recently published UK-FAST-forward data and the 26 Gy in 5 fraction regimen became a frequent option for early stage patients. This regimen balanced tumor control that is non-inferior to hypofractionation with decreased time in the hospital at a time when resources were short due to the COVID pandemic. While the weeklong FAST-forward (ultra-hypofractionation) has rapidly been adopted in countries like UK and SWEDEN, in USA the 3 weeklong FAST regimen (hypofractionation) remains the most frequent standard of care as adjuvant therapy after primary surgery for early breast cancer. Hesitation to utilize the FAST-forward regimen more frequently includes the short follow-up (5 years), as well as concerns regarding toxicity. The increased utilization of ultrahypofractionation for early stage breast cancer at MDACC during COVID was recently published, however, this is a preliminary report on differences seen in toxicity. Additionally, there have been anecdotal increases in toxicity overall for breast radiation patients in the COVID era. This summer, we did a preliminary study on the adverse effects of ultra-hypofractionation versus hypofractionation in the pre and post the COVID era to further understand the impact of fractionation schedule and COVID exposure on breast radiation toxicity

**Methods:** During the weeks and months after COVID, when healthcare resources were scarce, then recently published UK-FAST-forward data and the 26 Gy in 5 fraction regimen became a frequent option for early stage patients. This regimen balanced tumor control that is non-inferior to hypofractionation with decreased time in the hospital at a time when resources were short due to the COVID pandemic. While the weeklong FAST-forward (ultra-hypofractionation) has rapidly been adopted in countries like UK and SWEDEN, in USA the 3 weeklong FAST regimen (hypofractionation) remains the most frequent standard of care as adjuvant therapy after primary surgery for early breast cancer. Hesitation to utilize the FAST-forward regimen more frequently includes the short follow-up (5 years), as well as concerns regarding toxicity. The increased utilization of ultrahypofractionation for early stage breast cancer at MDACC during COVID was recently published, however, this is a preliminary report on differences seen in toxicity. Additionally, there have been anecdotal increases in toxicity overall for breast radiation patients in the COVID era. This summer, we did a preliminary study on the adverse effects of ultra-hypofractionation versus hypofractionation in the pre and post the COVID era to further understand the impact of fractionation schedule and COVID exposure on breast radiation toxicity. Oncora was used to identify patients with early stage breast cancer treated with lumpectomy and radiation over the designated time in the IRB approved protocol. Patient and tumor characteristics, as well as dosimetric data were gathered from the EPIC EMR. We used RedCap to record and verify the data. Statistical analysis was performed in SPSS

**Results:** Adverse effects were reduced in ultra-hypofractionation with  $p < 0.05$ . The Adverse effects studied using Fischer Exact test with  $p < 0.05$  were breast shrinkage, breast discoloration, breast edema, and breast pain at 6 months in addition to breast pain and dermatitis at the end of the treatment.

Conclusion: In our preliminary study of 300 records out of about 1000 patient records available Ultra-hypofractionation RT has a statistically significant ( $p < 0.05$ ) reduction of adverse effects in early stage breast cancer patients.

Keywords: Ultra-hypofractionation, Hypofractionation, Radiation, Adverse, Effect

Radiation Oncology Medical Student Summer Research Program

Abstract Number: 104

**A preliminary study of potential variations in toxicities from RT in early stage breast Cancer patients treated pre and post-COVID with attention to ultra-hypofractionation**

Revathi Gnanasekaran, Radiation Oncology, Foster School of Medicine Texas Tech El Paso

Background: During the weeks and months after COVID, when healthcare resources were scarce, then recently published UK-FAST-forward data and the 26 Gy in 5 fraction regimen became a frequent option for early stage patients. This regimen balanced tumor control that is non-inferior to hypofractionation with decreased time in the hospital at a time when resources were short due to the COVID pandemic. While the weeklong FAST-forward (ultra-hypofractionation) has rapidly been adopted in countries like UK and SWEDEN, in USA the 3 weeklong FAST regimen (hypofractionation) remains the most frequent standard of care as adjuvant therapy after primary surgery for early breast cancer. Hesitation to utilize the FAST-forward regimen more frequently includes the short follow-up (5 years), as well as concerns regarding toxicity. The increased utilization of ultrahypofractionation for early stage breast cancer at MDACC during COVID was recently published, however, this is a preliminary report on differences seen in toxicity. Additionally, there have been anecdotal increases in toxicity overall for breast radiation patients in the COVID era. This summer, we did a preliminary study on the adverse effects of ultra-hypofractionation versus hypofractionation in the pre and post the COVID era to further understand the impact of fractionation schedule and COVID exposure on breast radiation toxicity

Methods: We sought to assess the impact of COVID and implementation of ultrahypofractionation on acute and 6 month toxicity radiation therapy in patients undergoing treatment for early stage breast cancer. We reviewed 299 charts from patients treated from January 2019 date to September 2021 date treated with whole breast radiation with or without treatment to the low axilla. We excluded patients receiving treatment to the partial breast and patients receiving comprehensive nodal radiation. Demographic and treatment characteristics were extracted from the electronic medical record. We extracted the following characteristics: age, race, histology, T and N stage, modality, dermatitis, edema, fibrosis, breast shrinkage, breast discoloration, breast pain, ECOG, chemotherapy, hormone therapy, smoking history, cardiovascular diseases, COVID exposure, and COVID immunizations. We tabulated baseline patient and pathologic characteristics for all patients stratified by time and fractionation, hypofractionation prior to the pandemic, hypofractionation during the pandemic and ultrahypofractionation during the pandemic. 57 patients received up to 10 fractions of whole breast radiation, 243 patients received 11-28 fractions of radiation. 17 patients tested covid positive prior to radiation. 36 patients tested positive following radiation. 36 patients received COVID vaccination.

Results: Adverse effects were reduced in ultra-hypofractionation with  $p < 0.05$ . The Adverse effects studied using Fischer Exact test with  $p < 0.05$  were breast shrinkage, breast discoloration, breast edema, and breast pain at 6 months in addition to breast pain and dermatitis at the end of the treatment.

Conclusion: In our preliminary study of 300 records out of about 1000 patient records available Ultra-hypofractionation RT has a statistically significant ( $p < 0.05$ ) reduction of adverse effects in early stage breast cancer patients.

Keywords: Ultra-hypofractionation, Hypofractionation, Radiation, Adverse, Effect

Radiation Oncology Medical Student Summer Research Program

Abstract Number: 105

**Diagnostic performance of multiparametric MRI in radio-recurrent prostate cancer using the Prostate Imaging for Recurrence Reporting (PI-RR) and histopathological correlation**

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**Background:** Prostate MRI for Local Recurrence Reporting (PI-RR): International Consensus-based Guidelines on Multiparametric MRI (mpMRI) for Prostate Cancer Recurrence after Radiation Therapy were introduced recently. The purpose of our study was to assess their diagnostic performance using salvage prostatectomy-based histopathology as a reference standard.

**Methods:** This is a retrospective study of 52 consecutive patients with biochemical recurrence after external beam radiotherapy (EBRT). All patients underwent mpMRI between 2010 and 2020 followed by salvage prostatectomy for locally recurrent prostate cancer. Eight radiologists assessed the presence or absence of recurrent prostate cancer using PI-RR Guidelines after a dedicated training session. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and AUC of mpMRI per hemi-gland were calculated for each reader, and consensus read. A consensus read was considered positive if at least three independent readers agreed on a score. Inter-reader variability was assessed using Light's Kappa coefficient. p values of <0.05 were considered statistically significant.

**Results:** The median age of the patients was 66 years (range, 46-77). The mean PSA at the time of mpMRI was 4.1 ng/ml (range, 0.31-48). In 52 patients, histological findings were positive for recurrent disease in 92 hemi-glands and negative in 12 hemi-glands. Of those, 40 patients had the disease in bilateral hemi-gland, seven patients in left hemi-gland, and five in right hemi-gland. Sensitivity, specificity, PPV, NPV, and AUC of consensus read was 73.9%, 91.7%, 98.6%, 31.4% and 0.83 respectively. Inter-reader agreement was moderate (Light's Kappa = 0.59; P = 0.99).

**Conclusion:** Our study suggests that PI-RR International Consensus-based Guidelines have an overall good diagnostic performance for diagnosing recurrent prostate cancer in the setting of BCR following EBRT. Radiologists of varying experience demonstrated moderate agreement in detecting recurrence.

**Keywords:** MRI, Prostate, radiotherapy, recurrence

Summer Imaging Research Program

## Summer Imaging Research Program

Abstract Number: 106

### **Development of a “Universal” Phantom for Standardization of Chemical Exchange Saturation Transfer (CEST) MRI**

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**Background:** Chemical Exchange Saturation Transfer (CEST) MRI is an emerging molecular imaging method for improving cancer diagnoses. While a variety of image acquisition and analysis methods have been developed over the past twenty years, a standard phantom is needed to compare and standardize these various methods. Ideally, this standard phantom should contain a variety of materials and chemical agents that can exhibit a CEST effect to test the ranges of CEST image contrast that can be generated. CEST effect depends on temperature, necessitating accurate and precise control of the temperature of the standard phantom. Thus, our objective was to develop a “universal” standard phantom for CEST MRI at 3T and 7T magnetic field strength that consists of multiple materials that could exhibit CEST effect over a wide range of saturation frequencies while maintaining longitudinal stability and a steady temperature of 37.0 C during acquisition.

**Methods:** Base phantoms were obtained through purchase of a Diffusion Phantom from CaliberMRI and an Enhanced Multi Sample 120 Phantom from Gold Standard Phantom. Construction was achieved through removing the interior materials of these base phantoms, constructing a custom plate that could enhance the number of samples fitted, and filling with PureTemp37 (a liquid crystal that undergoes slow exothermic crystallization). Multiple samples of contrast, exogenous and endogenous agents were created with varying pH, R1 time, and concentrations. An MR spectrum was taken to verify temperature and homogeneity of sphere while the phantom was scanned in a 3T MRI to create B0, B1, T1 and T2 maps, as well as a CEST spectra of the samples.

**Results:** When measured over the course of six hours, the temperature of the isocenter, periphery and midpoint of the phantom stayed constant. Additionally, an MR spectrum was able to be successfully obtained. B0, B1, T1 and T2 maps were measured with both B0 and B1 showing homogeneity. The CEST spectra collected showed good CEST signal and that they were dependent on pH and T1 relaxation time.

**Conclusion:** Overall we were able to successfully design a physical phantom to capture the CEST effect and test the process of scanning the phantom. Further tests will be needed to ensure optimal parameters are created to best accomplish our goal of creating a “universal” phantom. Additionally, more work will be needed to validate the temporal stability of the phantom and determine optimal agents.

Keywords: Chemical Exchange Saturation Transfer (CEST), MRI Phantom, CEST effect  
Summer Imaging Research Program

Abstract Number: 107

### **Measuring the Effect of Myo-inositol Trispyrophosphate on Tumor Hypoxia with Multispectral Optoacoustic Tomography**

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Renee Chin, Cancer Systems Imaging, University of Texas MD Anderson Cancer Center

Mark Pagel, Cancer Systems Imaging, University of Texas MD Anderson Cancer Center,

**Background:** The hypoxic nature of solid tumors creates an environment that promotes tumor growth and development. Various factors contribute to the hypoxic tumor microenvironment leading to decreased T cell extravasation and increased T cell apoptosis. Because hypoxia hinders T cell trafficking and function in the tumor environment, the tumors are resistant to T cell-based cancer immunotherapies, leading to poor prognoses. Myo-inositol trispyrophosphate (ITPP) will be used to target hypoxia. ITPP increases oxygen unloading from hemoglobin, thus increasing %sO<sub>2</sub>. Multispectral Optoacoustic Tomography (MSOT) will be used to test if ITPP increased oxygenation within the tumor environment. MSOT provides a non-invasive way to measure tumor hypoxia to determine response to cancer therapies. MSOT measures deoxyhemoglobin and oxyhemoglobin at distinct wavelengths. The purpose of this study was to determine if ITPP improves oxygenation within the tumor environment through MSOT imaging to assess the potential of ITPP to improve response to immunotherapy.

**Procedure:** 3 days pre-implantation, 10 mice, 6 weeks of age, were shaved. 4T1 mammary epithelial tumor cells were implanted through a subcutaneous injection into the mammary fat pad. 9 days post-implantation, when the tumors were around 4 to 5 millimeters in diameter, baseline MSOT imaging was performed. 10 days post-implantation, 5 mice received ITPP injection while the other 5 mice received phosphate buffered saline (PBS) control injection. 3 hours post-injection, MSOT imaging was performed. **Methods:** Each mouse was anesthetized and given 21% medical grade air when put into the MSOT machine. The scan was run after the mouse equilibrated in the 37 °C water tank within the MSOT machine. MSOT emits light at different wavelengths (700, 730, 760, 800, 850, and 875 nm). The light is absorbed by oxyhemoglobin and deoxyhemoglobin, which undergoes thermoelastic expansion, also known as the optoacoustic effect. The thermoelastic expansion results in ultrasound waves. Images are created from ultrasound waves. After the images are obtained, spectral unmixing of the tumor image separates the oxyhemoglobin and deoxyhemoglobin absorption. %sO<sub>2</sub> ( $\%sO_2 = (HbO_2)/(Hb+HbO_2)$ ) is calculated, and the overall oxygenation of the tissue is obtained. The oxygenation levels from the baseline MSOT scan and post-ITPP injection scan are obtained and compared to determine if ITPP improved oxygenation. The process will be repeated with a PBS control injection instead of an ITPP injection to compare results.

**Results:** Through MSOT imaging analysis, we expect to see that ITPP improved oxygenation in tumors while PBS control did not.

**Conclusion:** We anticipate that there will be increased %sO<sub>2</sub> in the tumor microenvironment after ITPP injection. If the ITPP injection improves oxygenation in tumors, future studies will investigate the effect of ITPP on tumor size and immunogenicity. We expect to see that ITPP reduces tumor size and increases immunogenicity. Additionally, MSOT imaging is currently being tested within the clinical setting on human patients to assess hypoxia in tumors and predict response to therapy.

**Keywords:** Tumor hypoxia, ITPP, MSOT, Immuno-oncology, Checkpoint blockade

Summer Imaging Research Program



### **Irreversible Electroporation (IRE): Preclinical Assessment**

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**Background:** Irreversible electroporation (IRE) causes irreversible permeabilization of the cell membrane via short, high-voltage electrical pulses. This technique offers a promising treatment modality for local ablation of pancreatic ductal adenocarcinoma (PDAC). Our goal was to assess effects of IRE in vitro against various cell types present in the tumor microenvironment and in vivo in a preclinical liver metastasis model of PDAC.

**Methods:** To test the effect of IRE on cell proliferation, murine KRAS\* PDAC cells, murine RAW264.7 macrophages, and murine bone marrow derived macrophages (BMDM) were treated with IRE at 300 V or 400 V (20 pulses, 0.1 ms long, 1 second between pulses). Cell proliferation 24 hours after treatment was assessed with the MTS assay. Furtherly, the transwell migration assay was used to assess if IRE treated KRAS\* tumor cells affected macrophage migration. Immunofluorescence (IF) staining showed the density of CD169+ macrophages on KRAS\* tumor tissues from non-treated and IRE treated mice. Finally, PH252 cells (a variant of KRAS\* PDAC cells) were inoculated into the spleen of mice to develop a liver metastasis model of PDAC. Tumor growth was monitored by T2-weighted MRI. Statistical significance was determined by one-way ANOVA with  $p \leq 0.05$  considered statistically significant.

**Results:** IRE treatment decreased cell proliferation for all types of cells. KRAS\* cells were more sensitive to IRE treatment than both types of macrophages (RAW264.7 and BMDM). Increased IRE voltages were associated with increased cell death. IRE (400V)-treated KRAS\* cells significantly increased macrophage migration compared to untreated KRAS\* cells ( $p < 0.001$ ). In vivo model: IF staining of IRE-treated tumors revealed higher density of infiltrating CD169+ macrophages infiltration in IRE-treated tumors compared to non-treated tumors ( $p < 0.001$ ). Finally, MRI showed that injection of PH252 PDAC tumors into the spleen successfully metastasized to the liver, and the burden of liver metastasis (number of nodules and average size) was a function of time and number of tumor cells injected intrasplenically.

**Conclusion:** IRE potently decreased cell proliferation of tumor cells more than macrophages. IRE treated KRAS\* tumor cells promoted macrophage migration. This was confirmed with in vivo experiments, which showed increased tumor infiltration of CD169+ macrophages 24h after IRE treatment. Because CD169+ macrophages play an important role in antigen presentation, further studies on the effect of IRE on presentation of tumor associated antigens are warranted.

**Keywords:** Irreversible Electroporation (IRE), Pancreatic Ductal Adenocarcinoma (PDAC), Macrophage, Liver Metastasis

Summer Imaging Research Program

### **Correlation of Brain Dual Energy CT and Single Energy CT With Percent Hematocrit and Hemoglobin**

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**Background:** In CT imaging, certain materials can be made up of different elemental compositions but may possess the same CT numbers (Hounsfield Units, or HU). In order to differentiate these materials effectively, dual energy CT (DECT) has emerged as a viable solution. DECT uses a second energy to obtain attenuation measurements, which decomposes a mixture into its individual components and allows subsequent differentiation of the materials. DECT utilizes virtual monoenergetic images, which simulates images acquired with one precise energy from a range of 40-140 keV. This is beneficial as it allows one to optimize for maximum signal-to-noise ratio (SNR). A prior study investigated the relationship between SECT HU and percent hematocrit in the superior sagittal sinus (SSS). The results showed a statistically significant correlation between the two variables ( $r=0.5$ ). In our study, we sought to determine if this correlation improves using DECT.

**Methods:** Brain CT was acquired using a routine SECT protocol along with a fast kVp-switching DECT protocol for hemorrhage and calcification differentiation (Discovery 750HD, General Electric Healthcare, Waukesha, WI). The patient population ( $n=102$ ) was selected from a prospective clinical trial of DECT for hemorrhagic brain lesions. Patients were excluded for incomplete scans, incorrect scanning protocol, or misaligned SECT and DECT. Identical regions of interest (ROI) were placed within the SSS (AW Server, Version 3.2, General Electric Healthcare). Mean and standard deviation measurements were measured for DECT monoenergetic images (40-140 keV) and SECT. Complete blood count (CBC) measurements were evaluated if they occurred within 30 days of CT. SECT and DECT at 50, 65, 70, and 110 keV were correlated with percent hematocrit and hemoglobin.

**Results:** A total of 83 patients were analyzed in this cohort. SNR in the SSS peaked at 65-70 keV. The correlation of SECT HU to percent hematocrit was  $r=0.49$ , which was nearly identical to the literature value of  $r=0.5$ , thus validating our methods of ROI measurements. The correlation increases as energy increases, with both the 70 and 110 keV conditions ( $r=0.57$  and  $r=0.64$ , respectively) exceeding the SECT and literature values. The same trend is seen for DECT HU correlations with hemoglobin, with both the 70 and 110 keV conditions ( $r=0.57$  and  $r=0.65$ , respectively) exceeding the SECT correlation.

**Conclusion:** Overall, DECT via monoenergetic images shows promising potential for opportunistic neuroimaging applications. The results of this study may suggest future applications in detecting venous sinus thrombosis in the brain.

**Keywords:** Dual-energy CT, quantitative imaging, opportunistic imaging, hemoconcentration

Summer Imaging Research Program

## Summer Program in Cancer Research

Abstract Number: 110

### **Pol $\theta$ Protein Levels and Half-life in Lung Cancer Cells**

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**Background:** Lung cancer continues to be a major cause of death worldwide. Recently, lung cancer has been identified to display an over-expression of the DNA polymerase theta (around 83% of lung tumors). DNA polymerase theta (Pol  $\theta$ , coding by POLQ gene) is a main factor in the DNA double strand break repair pathway called theta mediated end-joining (TMEJ). The TMEJ pathway is an error prone pathway but essential to repair double strand break damage. Consequently, when Pol  $\theta$  is over-expressed, the activity of this pathway increases which may result in an increase in mutation levels and resistance to treatment. Lack of suitable antibodies and purified protein has hampered quantification of the expression of Pol  $\theta$  in human cells.

**Methods:** The first objective of my project was to determine the amount of Pol  $\theta$  in lung cancer cells. To determine the amount of Pol  $\theta$ , I compared Pol  $\theta$  protein levels of lung cell lines to purified full-length Pol  $\theta$  by immunoblot. The second aspect of this project is to determine the half-life of Pol  $\theta$  protein. The Pol  $\theta$  protein half-life is studied after blocking the protein production with cycloheximide treatment. The kinetic of cycloheximide treatment is analyzed by immunoblotting.

**Results:** The amount of Pol  $\theta$  depends greatly on the cell line and the cancer being studied. However, in over-expressed Pol  $\theta$  lung cancer cell lines, the average number of Pol  $\theta$  molecules per cell appears to be in the order of  $5.7E4$  molecules. The mean half-life of Pol  $\theta$  appears to be 21 hours. For the half-life of POL $\theta$ , we were able to observe two stages of degradation. First there was an increase in Pol  $\theta$  protein levels followed by a decrease in Pol  $\theta$  protein levels. This suggests that a factor involved in the degradation of Pol  $\theta$  has a short half-life, but further experiments are needed to get more precise results.

**Conclusion:** My project will support our understanding of the threshold where Pol  $\theta$  can have an activity in cancer cells. This research measured, for the first time, the level of Pol  $\theta$  in human cancer cell lines; as well as the half-life of Pol  $\theta$ . This sets the stage for future studies to analyze the mechanism of the degradation of Pol  $\theta$ . Knowing precisely the protein levels of Pol  $\theta$ , as well as its half-life, is essential to better understanding how Pol  $\theta$  may be inhibited in the fight against cancer.

**Keywords:** Pol  $\theta$ , TMEJ, DNA Repair, Lung Cancer

Summer Program in Cancer Research

Abstract Number: 111

### **Therapeutic potential of PRMT1 as a critical survival dependency target in multiple myeloma**

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**Background:** Multiple myeloma (MM) is a cancer of antibody-producing plasma B-cells and is the second most prevalent hematological malignancy worldwide. Intrinsic and acquired drug resistance impacts the therapeutic management of the disease and contributes significantly to treatment failure. This highlights a critical unmet need to identify novel vulnerability genes as therapeutic targets.

**Methods:** We recently identified Protein arginine N-methyltransferase 1 (PRMT1) as a top hit ( $p=0.0003$ ,  $FDR=0.01$ ) in a CRISPR/Cas9 based screen of 2000 sgRNAs targeting 200 DNA damage repair pathway related genes (i.e. 10 sgRNAs/gene) as a potential therapeutic vulnerability and survival dependency in MM cells. PRMT1 is a major Type I PRMT enzyme which catalyzes the asymmetric transfer of up to two methyl groups to arginine residues and regulates key cellular processes by modulating gene transcription and/ or protein functions via post-translational modification. PRMT1 dysregulation or overexpression has been observed in multiple solid and hematopoietic malignancies, and its overexpression is associated with cancer chemoresistance. Based on analysis of expression data from the Cancer Cell Line Encyclopedia (CCLE), PRMT1 appears significantly overexpressed in MM cell lines.

**Results:** We tested the recently developed Type I PRMTi GSK3368715 on a panel of MM cell lines and observed a dose dependent decrease in MM cells survival with IC50s within a range of 20 nM to 3  $\mu$ M for MM lines NCI-H929, MOLP8, KMS11, and U266. A decrease in the level of asymmetric dimethylation of arginine (ADMA) and accumulation of arginine monomethylation (MMA) was observed in PRMTi treated MM cells. In cell cycle analysis, we observed accumulation of cells in G0/G1 phase and simultaneous reduction of cells in S phase at 72 hours posttreatment. Further, the limiting dilution assay using different concentrations of PRMTi showed dose dependent reduction in cell proliferation.

**Conclusion:** Taken together, our preliminary data demonstrates that MM1 cells have a critical survival dependency on PRMT1 and shows the therapeutic potential of PRMT1 inhibition.

**Keywords:** Multiple myeloma, CRISPR/Cas9 screen, PRMT1, GSK3368715

Summer Program in Cancer Research

Abstract Number: 112

### **The Function of Arp5 in INO80 Nucleosome Remodeling Complex Histone Exchange Activity**

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**Background:** The INO80 nucleosome remodeling complex is responsible for genomic repair, recovery, and replication. It has been shown to repair double strand breaks and stabilize stalled or colliding replication forks by evicting RNAPII and preventing transcriptional machinery from collapsing the fork. Arguably the most important role of INO80 is its histone-exchange activity. INO80 evicts H2AZ histones and replaces them with H2A histones to stabilize the genome and facilitate progression of the cell cycle. In these experiments, we used the W303a strain of *Saccharomyces cerevisiae* to screen for Arp5 mutants in the acidic patch and DNA binding domain that restrict growth when under different forms of stress.

**Methods:** Using PCR, the Arp5 gene in each mutant strain was tagged with an FRB-KanMX6 cassette. Rapamycin was then used in spot assays to anchor the Arp5 gene out of the nucleus, and phenotypes were observed for multiple mutants. For FACS analysis, cells were grown with or without HU, and twenty-minute timepoints were recorded. RNase A and Proteinase K were used to digest the RNA and proteins before the cells were stained with Sytox Green. The samples were then analyzed in a flow cytometer.

**Results:** The phenotypes observed from the spot assays show P7 as the only mutant that is sensitive in low-phosphate conditions. This suggests that the ARP5 $\Delta$ 1 region may be involved in H2AZ and H2A dimer exchange. The P2 DBD mutant has a severe defect when combined with P7, a hydrophobic residue mutant, suggesting these residues have another function in Arp5 other than DNA binding in the INO80 complex. Due to the synergistic defect seen in the R2RNR strain, it is possible that the arginine anchor for the H2A acidic patch is among the R2 and RNR mutants. The P6 mutant showed sensitivity on both the DMSO and rapamycin plates which suggests that the mutant is dominant. The endogenous Arp5 is being affected while still in the nucleus.

**Conclusion:** The point mutations tested in this set of spot assays show that Arp5 acidic patch interaction could be the key to discovering how INO80 distinguishes H2AZ from H2A histones and carries out histone exchange.

**Keywords:** INO80, Arp5, H2AZ, nucleosome remodeling

Summer Program in Cancer Research

Abstract Number: 113

**MS-275 to modulate NK cell response against Osteosarcoma. Implication of the chemokines CXCL9/CXCL10**

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**Background:** Osteosarcoma (OS) is the most common primary malignant bone tumor in children and adolescents. It metastasizes almost exclusively to the lungs. Survival has remained stagnant for the last 30 years. Natural Killer (NK) cell therapy has shown benefit in the treatment of OS. However, the therapeutic effect of NK cells has been limited due to the inability of NK cells to penetrate to the tumor and this is in part attributed to the harsh immunosuppressive tumor microenvironment. MS-275, a histone deacetylase inhibitor, has preliminarily shown to enhance NK cell therapeutic efficacy against OS using an in vivo OS mouse model. However, it is unknown whether this effect is due in part to the ability of MS-275 to modulate the expression and/or release of the chemokines, CXCL9 and CXCL10, known to increase NK cell trafficking. Therefore, we aimed to determine whether pretreatment of NK cells with MS-275 influences the expression/release of these chemokines, hence contributing to the enhanced therapeutic efficacy seen in vivo.

**Methods:** Expression of CXCL9/CXCL10 on OS cells co-cultured with MS-275 treated and untreated NK cells was determined using immunocytochemistry and flow cytometry. Soluble CXCL9/10 was assessed using a chemokine array, and migration of MS-275 treated and untreated NK cells to OS cells was assessed using a transwell migration assay.

**Results:** Although exposure of NK cells to OS17 OS cells increases CXCL9/10 expression, epigenetic modulation of NK cells by MS-275 does not provide an additional increase on chemokine (CXCL9 & CXCL10) release and/or expression by OS tumor cells. Additionally, the migratory capacity of NK cells was not enhanced by pretreatment of NK cells with MS-275 ( $p= 0.6461$ ).

**Conclusion:** Our preliminary results indicate that exposure of OS cells to NK cells increases CXCL9/10 expression which will then contribute to migration and cytolysis of tumor cells. However, pre-treatment of NK cells with MS-275 does not trigger additional chemokine release/expression suggesting that these chemokines might not participate in the enhanced therapeutic efficacy of MS-275NK cells seen in vivo. Further studies are needed to validate these findings.

**Keywords:** Histone Deacetylase inhibitor (HDAC), MS-275, Entinostat, osteosarcoma, chemokines

Summer Program in Cancer Research

Abstract Number: 114

### **Targeting Arginine Methylation Reader SND1 in Acute Myeloid Leukemia**

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**Background:** Arginine methylation is an epigenetic hallmark of acute myeloid leukemia (AML). Staphylococcal nuclease and Tudor-domain containing 1 (SND1) is an arginine methylation reader that binds to symmetric di-methylarginine marks deposited by protein arginine methyltransferase 5 (PRMT5). Previous studies have shown that PRMT5 has an important role in AML development, and inhibiting PRMT5 reduces cancer cell growth in cell lines and mouse models of leukemia. We hypothesize that SND1 is similarly important to AML development and that its inhibition should also affect cancer cell morphology and growth.

**Methods:** We created stable and inducible SND1 knockdown lines for the MLL-ENL and NIH-3T3 cell lines. Western blot was used to assess the SND1 knockdown for each shRNA construct. Samples that showed SND1 knockdown were analyzed using Giemsa staining to determine changes in cancer cells (blasts).

**Results:** Two shRNAs in the pLKO.1 vector showed stable knockdown of SND1 in the NIH-3T3 and MLL-ENL cell lines. One shRNA in the pGIPZ vector showed knockdown in the NIH-3T3 cell lines but not in the MLL-ENL cell line. Giemsa staining showed terminal differentiation of blast cells in the two successful SND1 knockdown lines. For the inducible system, cell lines containing the successful shRNAs in the pTRIPZ inducible vector are currently being optimized for efficient growth so that SND1 knockdown efficiency and changes in cell morphology can be assessed.

**Conclusion:** SND1 has potential as a promising target for AML treatment. Stable knockdown of SND1 in a leukemia cell line disrupts the morphology of cancer cells. However, a successful inducible knockdown must be tested in a mouse model to verify this hypothesis.

**Keywords:** acute myeloid leukemia (AML), arginine methylation, protein arginine methyltransferase 5 (PRMT5), Staphylococcal nuclease and Tudor-domain containing 1 (SND1)

Summer Program in Cancer Research

Abstract Number: 115

### **Analyzing the Dynamics of MHV68 Uracil-DNA-Glycosylase in vitro and in vivo**

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**Background:** Epstein Barr virus and Kaposi sarcoma are both human gamma herpes viruses that target B cell germinal centers in lymphoid tissues. Each of these viruses are defined by lifelong infections and oncogenic properties. Part of its ability to infect for life is the lytic and latent phases that they undergo. The lytic phase of these viruses is defined by mass replication, while the latent phase, in which the infection spends much of the time, is composed of little to no replication except for sporadic lytic outbursts. Recently, a closely related KSHV homolog was found in rodents called murine herpes virus 68 (MHV68) which makes it an acceptable model for studying the dynamics of these related viruses in mice. MHV68 expresses its own distinct Uracil-DNA-Glycosylase (vUNG/ORF46) that helps prevent misincorporation of uracil into its DNA. Previous research implicated that vUNG is essential for the virus to establish latency in germinal centers (1), but the interactome and dynamics of expression for this critical gene is still not clear.

**Methods:** Study have determined that the lytic phase for MHV68 peaks at 14-16 days post infection in vivo (2). We utilized a plethora of experimental approaches to determine the detectable timeline for ORF46 expression. in vitro, we infected the virus in vivo, We infected mice intranasally under anesthesia as well as intraperitoneally at 100k PFU per mouse. These preliminary data will help future work on understanding the interactome and dynamics of vUNG/ORF46 during different phases of infection for these classes of viruses.

**Results:** in vitro, 3T12 fibroblast cells are YFP+ 12 hours post infection, and C1/Alexa647+ 24 hours post infection with peak lytic replication occurring at 48 hours. ORF46+ expression occurs at 8 days post infection in CD19+ cells in vivo with peak expression of not only MHV68+ germinal centers, but with CD19+ cells as well.

**Conclusion:** ORF46 expression occurs at an early stage (lytic phase) post infection in vitro. In vivo data suggests significant presence of ORF46 expression in CD19+ B cells at day 14, which is considered peak of lytic phase (3). ORF46 is important for maintaining the viral genome and may interact with other factors of the host machinery to assist in highly efficient viral replication. Further replicates are needed to confirm these results.

**Keywords:** Gamma herpesvirus, ORF46, oncogenic virus, MHV68, Uracil-DNA-Glycosylase

Summer Program in Cancer Research



Abstract Number: 116

### **Evaluation of a Nuclear Receptor-Binding SET Domain 2 (NSD2) Inhibitor for the Treatment of Leukemia & Brain Tumors**

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**Background:** Leukemia and brain tumors are the two most common pediatric cancers. Genome wide sequencing of these cancers has revealed mutations in epigenetic regulations that are unique to pediatric cancers, including mutations in the histone writer NSD2. NSD2 catalyzes the di-methylation of histone 3 lysine 36 (H3K36). An activating p.E1099K mutation was identified in acute lymphoblastic leukemia (ALL) and is associated with relapse. However, the role of NSD2 in other leukemias, such as acute myeloid leukemia (AML), has not been fully explored. Testing a NSD2 inhibitor on AML cell lines would reveal more about NSD2's role in AML. In addition, the brain tumor diffuse intrinsic pontine glioma (DIPG) harbors a mutation in histone 3 lysine 27 (H3K27) that limits methylation of H3K27 and promotes methylation of its antagonist H3K36, which is partially accomplished by NSD2. One study showed that knock down of NSD2 reduced tumor size of mice xenografted with DIPG, suggesting that a NSD2 inhibitor may be an effective treatment for these patients. However, the toxicity of a NSD2 inhibitor on normal brain cells, such as normal human astrocytes, has yet to be evaluated.

**Methods:** To determine the effect of the NSD2 inhibitor IACS-17596 on MOLM13 (human AML) cell proliferation and viability,  $83 \times 10^4$  cells per well were plated and then treated with 0.1  $\mu\text{M}$  - 10  $\mu\text{M}$  IACS-17596 the following day. Cell densities and viability percentages were then collected using a Beckman Coulter Vi-Cell Counter for eight days. In addition, alamar blue assays were performed to measure the effect of IACS-17596 on both MOLM13 and NHA cell viability. NHA cells ( $5 \times 10^4$ ) and MOLM13 cells ( $20 \times 10^4$ ) were plated and then treated with up to 50  $\mu\text{M}$  of IACS-17596 the following day. Alamar blue was added to the MOLM13s one day later and to the NHAs four days later, and then fluorescence was measured 16-18 hours later using the CLARIOstar microplate reader.

**Results:** During the few days following drug treatment, MOLM13 proliferation was slowed but viability was not affected. However, higher concentrations like 5  $\mu\text{M}$  or 10  $\mu\text{M}$  did eventually cause a decrease in viability, but this may have been due to non-specific effects of the drug. On the other hand, NHA cell viability was un-affected by concentrations of up to 20  $\mu\text{M}$ .

**Conclusion:** This compound demonstrates anti-proliferative effects on the MOLM13 cell line and is non-toxic on NHAs at low concentrations, encouraging future testing on leukemias and brain tumors, such as DIPG.

**Keywords:** Epigenetics, NSD2, MOLM13, NHA

Summer Program in Cancer Research

Abstract Number: 117

### **Probing the interaction of E2F1 & SART3, a novel arginine methyl reader**

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**Background:** Canonical functions of E2F1 (E2 promoter binding factor) are involved in transcription of genes and regulation of many cellular processes. Its involvement with RB (Retinoblastoma) can either promote or repress genes important for cell cycle progression depending on the activity of RB. In response to DNA damage E2F1 promotes DNA repair through its essential post-translational modifications including phosphorylation and acetylation. Reports on E2F1 methylation suggests that it also undergoes symmetric and asymmetric dimethylation at different arginine residues (SDMA and ADMA) during normal proliferation and genotoxic conditions. We are currently exploring the potential SDMA and ADMA sites of E2F1, their readers, and their biological functions. Recent work from our group found that SART3 (Squamous cell carcinoma antigen recognized by T-cells 3), is a new class of readers of SDMA. Here we study the interaction between SART3 and E2F1.

**Methods:** Our experiments used GFP-Trap immunoprecipitation (IP) assay in cells overexpressing EGFP-fused SART3 wild type (WT) or aromatic residue mutants. Then we treated the WT cells with PRMT5 inhibitor (PRMT5i) to know if the interaction is dependent on methylation.

**Results:** Our preliminary data suggests that SART3 interacts with E2F1. Data also suggests this interaction requires both aromatic residues of SART3 and symmetric arginine dimethylation of E2F1.

**Conclusion:** SART3 is likely a novel reader of methylated E2F1.

**Keywords:** Arginine methylation, E2F1, SART3

Summer Program in Cancer Research

Abstract Number: 118

### **Examining p53 binding to the promoter of ZNF365**

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**Background:** P53 is a tumor suppressor protein responsible for triggering various cell fates including cell cycle arrest and apoptosis. P53 has been known to bind to the promoter site of ZFP365, a mouse variant of the human ZNF365. Mutations in ZNF365 are associated with BRCA1 and BRCA2 mutations. The first aim of this experiment is to determine if p53 binds to the ZNF365 promoter, to then determine if p53 can be used to control expression of ZNF365. In many cancers, the gene encoding p53 is frequently mutated in some areas (hotspot mutations). The second aim is to understand how these hotspot mutations arise and why they are not fixed effectively by DNA repair mechanisms.

**Methods:** Nickel chromatography, ion-exchange chromatography, and size-exchange chromatography were primarily used to purify p53 DNA binding domain (p53DBD) and p53 core and tetramerization domain (p53CT) constructs. The protein-DNA interactions were measured by two methods: fluorescence polarization (FP) and electrophoretic mobility shift assay (EMSA).

**Results:** 9.64 mg/mL of p53DBD was purified (MW: ~25kDa). 0.341 mg/mL of p53CT was purified (MW: ~32kDa). The data shows that p53CT binds stronger than p53DBD to the p21 promoter at 200mM NaCl (pH = 7.5). Additionally, increasing ionic strength decreases the binding affinity of p53CT to p21. At a 200mM NaCl concentration (pH = 7.5), p53CT binds weakly to the ZNF365 promoter site, and p53CT binds weakly to coding regions of p53DNA and p53 T:G mismatch.

**Conclusion:** The singular base pair differences within the target sites of ZNF365 in comparison to those of p21 may be responsible for the weak binding of p53CT to the ZNF365 promoter. Additionally, the weak binding of p53CT to the coding region of p53 DNA and to the coding region of the p53 T:G mismatch may be explained by the 5 base pairs between the two target sites, compared to the 6 base pair difference between the p21 target sites.

**Keywords:** p53CT, p53DBD, ZNF365, p53DNA

Summer Program in Cancer Research

Abstract Number: 119

### **USP22 regulates EZH2 expression levels and binds its promoter**

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**Background:** USP22 (Ubiquitin specific peptidase 22), a histone deubiquitinating component of the SAGA complex, is one of the 11 “death-from-cancer” genes that is overly expressed in several aggressive cancer types. Our detailed analysis of the embryonic lethality observed in the Usp22 null mouse model we generated highlighted a critical role for Usp22 in the vasculature of the developing placenta. Usp22 null endothelial cells induced from mouse embryonic stem cells were unable to migrate and form vessels, possibly due to a defect in terminal differentiation. Endothelial cell differentiation and integrity has been linked to a functional enzymatic component of the Polycomb Repressive Complex 2 (PRC2), EZH2, a gene that provides instructions for repressive histone methylation. Since Usp22 expression is positively correlated with expression of members of the Polycomb group complex, we hypothesize that the levels of Ezh2 are affected in Usp22 null cells.

**Methods:** To investigate if the levels of Ezh2 are affected in Usp22 null cells, we used two main data components: RT-qPCR and ChIP-seq. We looked for amplification of the levels of Ezh2 in Usp22 null embryonic stem cells, which also consisted of testing other PRC2 complex subunits such as SUZ12, EED, BMI-1, and more. We aimed to identify the binding sites of Usp22 to map global binding sites of Usp22 in endothelial cells.

**Results:** We observed a significant reduction of both transcripts analyzed by RT-qPCR and EZH2 protein levels in the mutant endothelial cells, but no difference in the expression levels of EZH2 in Usp22 null mouse embryonic stem cells. We were unable to successfully perform a genome-wide USP22 occupancy analysis in endothelial cells (by ChIP-seq), however we were able to analyze the genome-wide recruitment of USP22 in mouse embryonic fibroblasts that express an overexpressed USP22 allele. We observed binding of USP22 protein to the promoter of EZH2, as well as the promoters of SUZ12 and EED, the obligatory binding partners of EZH2 in PRC2 complex.

**Conclusion:** Our results provide insights into an important role of USP22 in the regulation of EZH2 highlighting a potential functional interaction between the two proteins in the development of the vasculature.

**Keywords:** USP22, EZH2, PRC2.2 Complex, Embryonic Stem Cells.

Summer Program in Cancer Research

## Summer Undergraduate Research Program

Abstract Number: 121

### **Investigating Glucose Uptake Prognostic Effects On Pan-Cancer TCGA by METAFUX**

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**Background:** One major hallmark of cancer is deregulation of cellular metabolism. Higher uptake of glucose into cancer cells has been associated with more aggressive tumors. PET scans have been leveraged to infer changes in glucose metabolism, but pan-cancer glucose uptake studies for large cohorts are still lacking. To derive metabolic fluxes, we developed METAFux, which relies on three steps: calculating the metabolic reaction activity score (MRAS), flux balance analysis (FBA), and optimization of biomass reaction. Using METAFux, the objective of this specific study is to analyze 1) The association of predicted glucose uptake with clinical outcomes and 2) if these effects are homogenous across different types of cancer.

**Methods:** Using gene expression data from The Cancer Genome Atlas, METAFux calculated the MRAS using a specified set of gene-protein reaction rules and the Human-GEM, which contains information for 13082 metabolic reactions, 8378 metabolites, and enzyme relationships. METAFux then sets reaction and medium constraints for FBA analysis and runs quadratic programming using the OSQP package to minimize the sum of squared fluxes. We believe cells will minimize the total amount of enzymes to reach optimal growth. We then extracted glucose uptake data from the computed flux, sorting it into “High” and “Low” groups. Using the TCGABiolinks package, we analyzed the survival rates of both high and low glucose groups for every type of cancer.

**Results:** Thirty one different cancers were analyzed, showing whether glucose uptake levels had a significant effect on patient prognosis. Twenty-five showed an insignificant difference between high and low glucose uptake levels. In mesothelioma, lung adenocarcinoma, adrenocortical, and thyroid carcinoma cancers, survival for patients with lower glucose uptake was significantly higher. Meanwhile, in the brain lower grade glioma and thymoma cancers, analysis showed an opposite result, in that higher glucose uptake levels were associated with significantly higher survival.

**Conclusion:** Four different types of cancers are consistent with previous notions that higher glucose uptake is associated with more aggressive cancers and worse prognosis. However, two cancers contradict this and show that higher glucose uptake levels are significantly better. With the remaining cancers showing insignificant differences, the effect of glucose uptake on survival is non-homogenous among cancers. During the calculations, the mean was used as the benchmark for determining high versus low glucose uptake. Further studies with different benchmarks (median, top quartile, etc.) would be insightful. Using METAFux, we have identified six different cancers of interest to analyze further.

**Keywords:** METAFux, Pan-Cancer, Glucose uptake, Metabolic reprogramming, TCGA

Summer Undergraduate Research Program

Abstract Number: 122

### **KMT2D Loss Promotes Head and Neck Squamous Cell Carcinoma Through modulation of Immune Microenvironment**

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**Background:** Head and neck squamous cell carcinoma (HNSCC) is the sixth most common type of cancer in the world. Epigenetic factors have been acknowledged as the gatekeepers of crucial homeostatic events in the normally functioning cells. Thus, aberrations in the epigenetic machinery are known to contribute to tumorigenesis in various cancer settings. Here we focus on KMT2D protein, a major mammalian histone 3 lysine mono methyltransferase. It has been shown to act as tumor suppressor in various cancer models. This study investigates KMT2D as a tumor suppressor factor in HNSCC and discusses its role in regulating tumor-intrinsic antigen presentation machinery.

**Methods:** Clinically relevant data was acquired from publicly available TCGA dataset and processed for further analysis. Non-targeted (NT) and Knockdown (KD) KMT2D HN30 HNSCC cell lines were generated and subjected to RNAseq and Western blotting analysis to assess the terms associated with antigen processing and presentation machinery. In vivo experiments were conducted on C57BL/6J mice utilizing NT and KD KMT2D MOC1 DOX-inducible cell lines to orthotopically implement tumors into tongues.

**Results:** The analysis of TCGA datasets revealed that KMT2D mutations were observed in 17% of HNSCC cases. KMT2D mutations were correlated with lower recurrence-free rate survival after two years. KMT2D knockdown cell lines had decreased mRNA levels of immune-associated factors, such as interferon regulatory factors (IRFs) and groups of cytokines and chemokines. In addition, the protein and mRNA levels of two antigen-processing machinery members, TAP2 and ERAP1, were reduced in KMT2D KD HN30 cell lines. The data collected from our syngeneic mouse model demonstrated a higher tumor burden in the KMT2D-deficient group.

**Conclusion:** These findings show that KMT2D is a major epigenetic factor controlling HNSCC onset and give opportunities to explore novel treatment plans with immunomodulatory approaches for patients harboring KMT2D loss-of-function mutations.

**Keywords:** KMT2D, HNSCC, antigen processing and presentation machinery, TAP2, ERAP1

Summer Undergraduate Research Program

## **Prediction of Liver Regeneration Post-Radiotherapy Using Machine Learning and Deep Learning Models**

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**Background:** Risk of hepatic failure due to limited functional liver volume after radiotherapy (RT) can be minimized when there is an accurate prediction and incorporation of RT induced liver hypertrophy during RT treatment planning. However, current models lack the response prediction at the segment level. Therefore, we aimed to identify the predictors responsible for segmental hypertrophy and developed machine-learning (ML) and deep-learning (DL) models to predict liver segment hypertrophy.

**Methods:** Computed tomography scans of 105 patients treated for liver cancer at MD Anderson Cancer Center were obtained. 122 features including clinical and dosimetric parameters were collected. Volumetric change in liver segments 1, 2, 3, 4, 5-8, and 2-3 were estimated at three-month follow-up and were binarized into hypertrophy and non-hypertrophy outcomes. Univariate analysis (UA) was performed to remove non-significant variables ( $p > 0.2$ ). For categorical variables, Chi-squared or Fisher's exact tests were used according to expected frequency size. Simple Logistic Regression was used for numerical variables. Pearson Correlation ( $\rho > |0.75|$ ) was used to eliminate collinearity among variables. The resulting dataset were held for ML training. Multivariable analysis (MA) was performed by multiple logistic regression with backward feature selection to identify relationships among variables. ML models using Lasso Regression (LR) and Random Forest (RF) (10-fold cross validation, TR=81, T=24) were separately trained for hypertrophy prediction of each segment. Data were resampled using upsampling, downsampling, and Random Over-Sampling Examples (ROSE) for segments with imbalance ratio  $> 2$ . Further, a single-layered feedforward neural network (10-fold cross validation, TR=80, T=25) with ROSE was trained on all variables. Accuracy, receiver operating curve (ROC), area under the curve (AUC), sensitivity, specificity were evaluated for each model.

**Results:** MA demonstrated that tumor in segment 5-8 was protective factor and percent volume receiving dose  $< 15\text{Gy}$  in segment 1 was risk factor for segment 1 hypertrophy. Tumor in segment 5-8 was risk factor for segment 3 hypertrophy. Tumor in segment 4 was protective factor for segment 4 hypertrophy. Portal vein thrombosis was protective factor for segment 5-8 hypertrophy. Overall, LR with ROSE and RF with upsampling showed the highest test accuracies and AUC for all segments. RF showed 100% train accuracy while LR with ROSE showed 73% average train accuracy. DL showed superior performance than ML for segment 1.

Conclusion: Tumor locations and dosimetric variables were significant protective/risk factors for MA and important predictors of liver hypertrophy in all ML and DL models. Overall, ML models showed superior results than DL models.

Keywords: Liver Regeneration, Radiotherapy, Prediction, Machine Learning, Deep Learning

Summer Undergraduate Research Program



Abstract Number: 124

### **Targeting Polo-Like Kinase 4 (PLK4) Triggers Polyploidy and Apoptosis in TP53-mutant Acute Myeloid Leukemia and Results in Improved Survival**

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**Background:** TP53 mutations in acute myeloid leukemia (AML) are associated with complex karyotype and a high risk of relapse. While novel treatment regimens, including the combination of the BCL2 inhibitor venetoclax (VEN) and hypomethylating agents (HMA), have emerged as partially effective treatments and resulted in higher remission rates in patients with TP53-mutant AML, clearance of the mutant TP53 clone is rarely achieved and the majority of patients relapse. The mechanisms responsible for relapse in TP53-mutant AML remain unclear and investigating novel mechanisms is critical to developing more effective therapies.

**Methods:** In order to investigate defective signaling pathways in TP53-mut AML, we have analyzed two clinically annotated (karyotype, survival, complete blood counts, and previous treatments) RNA-sequencing datasets: MDACC AML Moonshot dataset included 19 TP53-mutant and 25 TP53-wt samples, and the Munich Leukemia Laboratory (MLL) included 72 TP53-mutant and 654 TP53-wt samples. For in vitro experiments, we have utilized TP53-mut CRISPR-generated MOLM13 cells. For in vivo models, we have transplanted TP53-mut MOLM13 AML cells into NSG mice (n=58: untreated n=28, treated n=30), and treated with CFI-400945 (oral gavage 7.5 mg/kg, 5 times/week) for 3 weeks.

**Results:** Using the Moonshot dataset, we identified Polo-like kinase 4 (PLK4), a key regulator of centriole biogenesis, as a potential target highly expressed in TP53-mutant AML samples ( $>2$  log<sub>2</sub>-fold change,  $p=0.0009$ ). We validated our finding utilizing MLL dataset and confirmed that TP53-mutant AML samples consistently showed PLK4 upregulation ( $p=0.0003$ ). Additionally, PLK4 protein levels were significantly higher in TP53-mutant AML MOLM13 cells when compared with TP53-wt AML MOLM13 cells. Experimentally, we found that PLK4 inhibition for 72 hours using 25nM CFI-400945 in TP53-mutant AML MOLM13 cells triggers polyploidy ~2-fold higher than in TP53-wt AML MOLM13 cells ( $p<0.0001$ ). Furthermore, our data suggested that polyploidy is not reversible after drug removal and resulted in increased levels of apoptotic cell death in TP53-mutant AML MOLM13 cells. To translate our findings to in vivo models, we showed that PLK4 inhibition in mice transplanted with MOLM13 cells have improved overall survival compared to untreated mice (20.7%, 25%, and 37.5% overall survival increase for TP53-wt TP53-mut, and TP53-ko respectively).

**Conclusion:** Our data suggest that TP53-mutant AML expresses higher levels of PLK4 compared to TP53-wt AML and targeting PLK4 triggers polyploidy and apoptosis in TP53-mutant AML. We show that PLK4i-induced apoptosis translates to improved survival using in vivo models. TWT-202 is an ongoing clinical trial testing the efficacy of PLK4i in AML.

**Keywords:** AML, PLK4, p53, Apoptosis, Polyploidy

Summer Undergraduate Research Program

Abstract Number: 125

### **Role of ASH1L in Prostate Cancer Metastasis**

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**Background:** ASH1L is a histone lysine methyltransferase that catalyzes the dimethylation of histone H3 at lysine 36 (H3K36). ASH1L regulates gene expression in leukemia and plays a role in acute leukemia progression and poor prognosis. The overexpression of ASH1L was also found in breast, liver, and thyroid cancer and has been linked with increased cell cancer growth and disease aggression. However, the activity of ASH1L has not been explored in prostate cancer (PCa) metastasis.

**Methods:** Data for bioinformatics analysis was gained from The Cancer Genome Atlas primary PCa and SU2C metastatic PCa patient samples. Cell migration and 3D invasion assays were performed using control and ASH1L knockout (KO) PC3M cells. Analysis of invasion assay was conducted with NIH ImageJ software. Real-time PCR (qPCR) was performed using SYBR Green reagent (Applied Biosystems) in control and ASH1L overexpressed LNCaP cells. Immunofluorescence (IF) was performed in bone sections of DX1-derived syngeneic bone metastasis model (intratibial injection) and detected using a VectraPolaris scanner (PerkinElmer). Western blots were performed to determine expression of ASH1L, methylations of H3, and EMT transcription factors.

**Results:** Multi-omics analysis of human PCa samples showed ASH1L gene amplification and gain are more frequent in metastatic PCa than in primary tumors. Patients with ASH1L amplification exhibited much shorter overall survival than those with ASH1L diploid, indicating a role in metastasis. In addition, migration assays and 3D invasion assays were performed with PC3M control and ASH1L KO cells. Migration assays showed significantly lower migration capacity of ASH1L KO cells. Invasion assays revealed that ASH1L KO cells have little to no invasion activity, whereas control cells have 7 times longer invasion lengths and 35 times larger areas than KO cells. Furthermore, we found that ASH1L overexpression led to increased expression of SNAI1 and SNAI2, both are involved in the epithelial-mesenchymal transition (EMT) process. Genes in the matrix metalloproteinases (MMPs) family (involved in metastasis) were also upregulated upon ASH1L overexpression, implicating ASH1L's role in mediating cell migration.

**Conclusion:** The histone lysine methyltransferase ASH1L gene is frequently amplified in metastatic PCa, and high expression levels of ASH1L are associated with worse patient survival. Our study uncovered that ASH1L induced cell migration and invasion, partially due to the upregulation of pro-metastatic genes, such as MMPs and EMT-related transcription factors. Collectively, these results suggest that ASH1L plays a metastasis-promoting role in PCa and thus could be a therapeutic target for metastatic PCa.

**Keywords:** Histone methyltransferase, epithelial-mesenchymal transition, metastasis, ASH1L, prostate cancer

Summer Undergraduate Research Program

Abstract Number: 126

### **Synthesis of Block Copolymers to Deliver Ortho-Carborane for Proton Capture Therapy**

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**Background:** In the last several decades, conventional drug delivery has been intensively researched utilizing different delivery vehicles, such as nanoparticles, polymers, and conjugated antibodies. However, medication leakage in the internal organs remains one of the field's greatest obstacles. Utilizing the unique features of protons, irradiation treatments such as proton therapy could deliver precise treatment to the intended target. However, the current proton therapy strength could be further improved to eliminate the tumor effectively. We propose delivering ortho-carborane to the tumor via polymer micelles to augment proton therapy.

**Methods:** In order to encapsulate the boron compound, multiple block copolymers are being synthesized to create polymer micelles of varying sizes. This work illustrates the synthesis of various sizes of poly(PEGMA)<sub>n</sub>-b-poly[HEMA-g-( $\epsilon$ -caprolactone)]<sub>m</sub> in order to assess their stability and capacity to encapsulate and release ortho-carborane. The boron cargo will be harmless to all cells and can only be activated to eradicate cells via proton capture therapy, allowing for precision targeting and safety to other internal organs.

**Results:** Poly(PEGMA)<sub>n</sub>-b-poly[HEMA-g-( $\epsilon$ -caprolactone)]<sub>m</sub> in different sizes were successfully synthesized. Also, they are capable of self-assembling to form nanoparticles in water. In addition, the dynamic light scattering results demonstrates that the concentration of the polymer does not affect the size of the nanoparticles.

**Conclusion:** The block copolymers have been confirmed to be a viable delivery vehicle for ortho-carborane. Further studies are currently under investigation for the optimal block copolymers configuration that could maximize the ortho-carborane loading capacity and its release profile at the tumor site.

**Keywords:** Polymer micelles, Proton capture therapy, Drug delivery

Summer Undergraduate Research Program

Abstract Number: 127

## **Evaluating the Reproducibility of Gene Regulatory Networks Construction Using Biological Replicates**

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**Background:** This study aimed to determine whether the single-cell gene regulatory networks generated by SCORPION, a tool developed in Yi's lab at UT Austin, could be replicated using two samples from different parts of the same polyp. Using single-cell RNA-seq, the expression of the genes in each sample was characterized, and this information was used to construct independent gene regulatory networks with SCORPION. Our working hypothesis was that SCORPION would be able to accurately and consistently reconstruct the gene regulatory network from multiple biological samples and replicates. Ultimately, this is significant for future population-based studies because it facilitates the linking of transcription factors to the target gene they affect in the design of pharmacological agents.

**Methods:** For this experiment, two publicly accessible single-cell RNA-seq datasets from the same donor were downloaded from the GEO database. We combined the data using Harmony and labeled the various cell types on a UMAP low-dimensional representation with canonical markers. We selected T-cells from both samples for the construction of gene regulatory networks. To assess the reproducibility of the gene regulatory networks, a correlation analysis was performed on the weights of the edges. R was utilized for all analyses.

**Results:** The GSM6061664 (9591 cells) and GSM6061665 (2243 cells) samples were downloaded and loaded into R. After passing quality control, 11834 cells were retained and incorporated using Harmony into a UMAP low-dimensional representation. We identified 18 cell types in the samples, which corresponds to the number reported by the dataset's authors. We chose CD3D T-cells in order to construct gene regulatory networks accounting for the interaction of 622 transcription factors and 15,493 genes. The weights of the edges (9,636,646 in total) between the gene regulation networks of the two samples were then compared. We found that their Pearson's correlation coefficient was 0.913 and their p-value was 2.2e-16.

**Conclusion:** This positive and statistically significant result provides evidence that the gene regulation networks of the different sections of the polyp were very similar, suggesting that SCORPION will be able to construct gene regulation networks that are very similar regardless of where the sample is taken, ensuring that the gene regulation networks can be reproduced using biological replicates.

**Keywords:** Colorectal Cancer, Gene Regulatory Networks, Single-Cell RNA-seq, SCORPION Program

Summer Undergraduate Research Program

Abstract Number: 128

### **Distinguishing between Noncancerous Neural Stem Cells and Neural Tumor Stem Cells**

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**Background:** Glioblastomas are exceedingly aggressive brain tumors. Inside glioblastomas, cancer stem cells with the potential to advance tumor resistance, development, and recurrence have been detected. While noncancerous stem cells are the building blocks of all organs and tissues, cancerous cells with stem-like qualities have posed a considerable challenge in tumor eradication. SOX2 is a stem cell marker expressed in noncancerous neural stem cells, while  $\beta$ -III tubulin and synapsin are differentiation markers. We hypothesize that GSCs express abnormal levels of stem cell markers and differentiation markers when compared to NSCs. To test this hypothesis, the expression levels of synapsin, SOX2, and  $\beta$ -III tubulin were analyzed in GSCs and NSCs.

**Methods:** GSCs were obtained by dissociating a tumor sample removed through a patient receptive surgery, selecting single tumor cells, and then culturing the sample. NSCs were derived by removing part of the frontal cortex of mice embryos on the 12th day of development and then culturing until the sample contained both NSCs and neurons. A western blot was conducted and the protein expression levels in each cell type for synapsin, SOX2, and  $\beta$ -III tubulin were quantified using actin as a control.

**Results:** The western blot results show a more than 10-fold decrease in SOX2 protein expression in GSCs versus NSCs. Additionally, GSCs showed an 8-fold reduction in the protein expression of  $\beta$ -III tubulin compared to the NSCs, and GSCs had more than an 88-fold decrease in the synapsin protein expression when contrasted with the NSCs.

**Conclusion:** In accordance with our hypothesis, our findings show that GSCs express abnormal levels of stem cell markers and differentiation markers when compared to NSCs. Given that  $\beta$ -III tubulin and synapsin markers are present when early and late differentiation have transpired, one would expect these expression levels for GSCs to be low since cancer cells do not differentiate appropriately. Finally, considering that SOX2 is an NSC marker, it is logical for SOX2 expression to be almost nonexistent in GSCs. The fact that the GSCs had low  $\beta$ -III tubulin and SOX2 expression levels and a negligible level of synapsin suggests that the GSC line utilized possesses markers of both stemness and differentiation and displays abnormal differentiation properties. Further investigation is needed to investigate the mechanisms that make the expression levels of these stem cell markers and differentiation markers different in cancer stem cells versus noncancerous stem cells.

**Keywords:** stem cells, glioblastoma, SOX2, synapsin,  $\beta$ -III tubulin

Summer Undergraduate Research Program

### **Targeting AXL with a Highly Stable Modified Aptamer in Medulloblastoma Cell Lines**

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**Background:** Medulloblastomas are the most common types of pediatric brain tumors and start in the cerebellum. They represent approximately 20% of pediatric brain tumors, with an average age of diagnosis between 5 and 9 years old. Surgery followed by radiation, and chemotherapy is the standard treatment, however, it can be ineffective due to the location of the tumor and the age of the patient. Thus, patients would benefit from non-toxic, targeted therapeutics. One approach is to use aptamers, synthetic, single-stranded RNA or DNA oligonucleotides that selectively bind their target proteins with high affinity. Aptamers are considered chemical antibodies that are non-immunogenic and easily penetrate the tumor site due to their small size. A promising target for aptamer-induced inhibition is AXL, a receptor tyrosine kinase overexpressed in many types of cancers and associated with poor patient survival. The main ligand of AXL is Gas6, and the AXL/Gas6 signaling pathway has been shown to enhance proliferation, invasion, metastasis, and drug resistance. Inhibiting AXL activation may therefore provide a therapeutic effect, and we have reported that the highly stable and modified GLB-A04 aptamer inhibits phosphor-AXL in vitro in Gas6-stimulated medulloblastoma cell line.

**Methods:** We examined AXL expression and its effect on survival in brain tumor patients using expression and survival databases. To determine whether GLB-A04 can reduce the expression of the active form of AXL, we treated Daoy cell line with GLB-A04 and Gas6. We also performed Annexin V and Western blot to determine the effect of GLB-A04 in apoptosis.

**Results:** Here, we find that AXL is highly expressed in medulloblastoma cell lines and brain tumors, and high expression is associated with lower patient survival. Interestingly, we find no difference in cell viability after 72 hours treatment with concentrations of 1.6  $\mu\text{M}$  of GLB-A04 compared with scramble aptamer used as negative control. Additionally, we find no difference in the expression of Caspase 9, Cleaved caspase 9, Caspase 8, and Cleaved PARP.

**Conclusion:** Lower survival in patients expressing high levels of AXL makes it a potentially viable therapeutic target in the treatment of brain cancer. Additionally, the enhanced stability of the GLB-A04 aptamer and strong binding to AXL have been shown to inhibit phospho-AXL in vitro. Here, although we find no difference in cell viability or apoptotic protein expression, experiments need to be repeated with higher concentrations of aptamer and longer treatment times.

**Keywords:** Aptamers, AXL, targeting therapy, Medulloblastoma

Summer Undergraduate Research Program

### Probing Spatial Myeloid Heterogeneity in Glioblastoma

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**Background:** Conventional adaptive immunotherapy is ineffective against glioblastoma multiforme (GBM) due to factors like T cell exhaustion. Modulating pro-tumor brain myeloid cell activity would present a viable alternative. Due to ethical considerations, murine models are better suited than human samples for investigating myeloid heterogeneity in whole brain tissue. Our immunocompetent GBM model—*Qk<sup>L/L</sup>;Pten<sup>L/L</sup>;Trp53<sup>L/L</sup> (QPP)*—more faithfully reproduces the immune environment of human GBM than does *GL261*, the most common preclinical GBM model. Thus, we leveraged *QPP* to study myeloid composition across tumorigenic brains. We probed cell morphology, distinguished resident microglia from circulation-derived macrophages (CDMs), and examined for pro- or anti-phagocytic markers.

**Methods:** We generated *QPP7* GBM from *Nestin-CreER<sup>T2</sup>;QPP* mouse injected with tamoxifen at P7. To generate syngeneic *QPP7* GBM, cells from *QPP7* cell line were implanted intracranially into B6 *Cx3cr1-CreER<sup>T2</sup>;mTmG* mice. Brains were harvested from moribund mice, fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 5  $\mu$ m via microtome for immunofluorescence. Sections were stained/co-stained with 1:250 primary antibody dilution and 1:1000 secondary antibody dilution (488/594 nm) and imaged via widefield microscopy.

**Results:** Iba1 staining revealed four morphotypes with undiscovered functions. Ramified Iba1<sup>+</sup> cells, abundant in non-tumor tissue, have long processes characteristic of homeostatic microglia. Hyper-ramified and rod Iba1<sup>+</sup> cells, found mostly at the tumor border, strongly express Iba1 and have fewer processes. Activated Iba1<sup>+</sup> cells, found increasingly towards the tumor core, lack processes and could be resident microglia or CDMs. We co-stained for microglial marker TMEM119 but found a lack of TMEM119 expression in all Iba1<sup>+</sup> cells, indicating TMEM119's downregulation in glioma conditions. Iba1<sup>+</sup> CD45<sup>high</sup> cells, or likely CDMs, were found increasingly towards the tumor core. Tumor cells largely expressed CD47, indicating phagocytic suppression. Arg1, marking inflammation suppression and tissue healing, was expressed by mostly tumor associated Iba1<sup>+</sup> cells. Co-staining spontaneous *QPP* tumor sections for Iba1 and GFP, a reporter signal for *Qki*-null tumor cells, we discovered phagocytic uptake only in some border regions. It remains unclear *when* phagocytosis occurred and if uptake and/or endolysosomal activity were diminished.

**Conclusion:** Microglia play some role in defending against or promoting gliomagenesis. Localized or morphotype-dependent microglial gene signatures can be investigated via fluorescence-activated cell sorting and single-cell RNA sequencing. In glioma conditions, there are phagocytic suppression and high CDM trafficking into the tumor. Both can be probed further via secretomics, lineage tracing, *in vivo* tracking, and time course studies. Multiplex immunofluorescence and confocal microscopy would allow stronger visualization of tumor-burdened brains.

**Keywords:** GBM, microglia, CDM, phagocytosis, Iba1.

Summer Undergraduate Research Program

Abstract Number: 131

### **Identifying Biomarkers to Select Patients with Borderline Resectable and Locally Advanced Pancreatic Ductal Adenocarcinoma (BRPC, LAPC) for Radiotherapy (RT)**

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**Background:** BRPC and LAPC have poor prognosis in which surgery is the only curative treatment. RT has a controversial role for BRPC and LAPC, with negative and positive data in recent years. There is an unmet need to identify biomarkers to select subpopulations of patients for RT. Prior results indicated that CA19-9 response and lymphopenia grade associate with outcomes after RT. Here, we investigated these variables and other clinical factors to identify biomarkers that may aid the decision to use RT for BRPC and LAPC.

**Methods:** We conducted a retrospective study to analyze patients who received chemo followed by RT for BRPC or LAPC between 2015 and 2020. CA19-9 normalizers were defined by the minimum CA19-9 value between the start of chemo and 6 months post-chemo that was  $< 40$  U/mL. Patients were identified as having a lymphopenia grade  $>2$  if their ALC fell below 0.5 K/uL during RT. Associations between variables were tested using Log-rank and Wilcoxon survival analyses. Variables with a p-value less than 0.2 in univariate analyses were then used in a multivariate Cox Proportional Hazard test.

**Results:** 233 patients were found (108 F, 125 M, median age 67 [30-86]) who received RT for BRPC or LAPC. Median OS for all patients from date of RT was 12.4 months (range, 0.5-65.8 months). CA19-9 normalization was associated with OS, as median OS from date of RT for CA19-9 normalizers and non-normalizers were 19.3 months and 11.5 months, respectively ( $p = 0.015$ ). When analyzing patients who were non-normalizers, patients who received surgery had a mean OS of 25.4 months vs. 8.84 months for those who didn't ( $p = .0001$ ). For patients with a lymphopenia grade  $>2$ , patients with BRPC had a median OS of 23.4 months compared to LAPC with 11.6 months ( $p = 0.003$ ). Type of radiation was also found to be associated with overall survival, with Stereotactic Body Radiation Therapy having the highest median OS of 22.7 months and Intensity-Modulated Radiation Therapy having the lowest median OS of 10.1 months ( $p = 0.019$ ). Associated variables were ran in a Cox Proportional Hazard test in which CA19-9 normalization ( $p = .00012$ ), radiation type ( $p = .0032$ ), and surgery vs. no surgery ( $p = .0001$ ) were statistically significant.

**Conclusion:** Variables associated with higher OS in analyses could be used as potential biomarkers to select patients for RT. Additional prospective trials are needed to evaluate the ability of these factors to personalize treatment and solidify stable biomarkers.

**Keywords:** Pancreatic Ductal Adenocarcinoma, Radiotherapy, Biomarkers, CA19-9, Lymphopenia

Summer Undergraduate Research Program



Abstract Number: 132

### **Cell Differentiation: The Case of Turning THP-1 Cells into M1 Macrophages**

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**Background:** The Calin lab focuses on the role of miRNAs, small non-coding RNAs that regulate gene expression, in cancer. THP-1 is a cell line of monocytes, a type of white blood cell, from an acute myeloid leukemia patient. The goal of this project is to differentiate monocytes into M1 macrophages, a type of macrophage that inhibits tumor growth, and characterize the macrophages based on the markers and miRNAs they express.

**Methods:** THP-1 cells were cultured and differentiated into M0 macrophages with phorbol 12-myristate 13-acetate (PMA). Cells were then activated into M1 macrophages using lipopolysaccharide (LPS) at concentrations of 1, 10, and 20 ug/mL for 6 and 24 hours. An MTS assay was done to test the cell viability after treating with LPS. Total RNA was extracted from treated and control cells and the concentration was analyzed using Nanodrop spectrophotometer. Total RNA was reverse transcribed into cDNA and RT-qPCR was used to analyze the expression level of differentiation markers (IL 1 $\beta$ , IL 12 $\alpha$ , and IFN  $\gamma$ ) and miRNAs (miRNA 125A, miRNA 146A, and miRNA 155) associated with LPS induced M1 macrophages.

**Results:** After differentiating with PMA into M0 macrophages, the cells attached after 2 hours and changed morphology after 72 hours. The MTS Assay showed that cell viability does not significantly decrease by treating cells with LPS. For the specific genes used to characterize macrophages, only the 10 ug/mL LPS for 6 hours and 1 ug/mL for 6 hours treatments showed at least two out of three significant increases in gene expression level. The other treatments showed only one significant increase, no significant changes, or all significant decreases in gene expression level. For the miRNAs associated with M1 macrophages, only the 20 ug/mL LPS for 24 hours treatment showed at least two out of three significant increases in miRNA expression level. The other treatments showed either no significant changes or at least one significant decrease in miRNA expression level.

**Conclusion:** I concluded that the cells differentiated into M0 macrophages. The MTS assay shows that LPS does not affect cell viability. For the macrophage genes, I concluded that the 10 ug/mL for 6 hours and 1 ug/mL for 24 hours treatments worked best. This means that the activation into M1 macrophages is both time and dose dependent. The miRNA expression is also time and dose dependent as only the 20 ug/mL LPS for 24 hours showed increased expression level of the miRNAs.

**Keywords:** Macrophages, Differentiation, THP-1, miRNA

University Outreach - Bryn Mawr College

### **The Role of Exosomal miR-181c-3p Within the Ovarian Tumor Microenvironment**

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**Background:** Annually, 21,000 women are diagnosed with epithelial ovarian cancer (EOC) in America. Most of them are short term survivors (STS) who live for less than two years after the initial therapies, including debulking surgery and chemotherapy, and few are long-term survivors (LTS), surviving over seven years after treatment. Recent studies showed that cells in the tumor microenvironment (TME) could communicate through exchange of exosomal microRNAs (miRs) to modulate the TME and ultimately affect patients' survival. Previous miR sequencing analysis on formalin-fixed paraffin-embedded (FFPE) tumor tissue samples from LTS and STS of high-grade serous ovarian cancer (HGSC) patients resulted in a signature of 50 miRs that were expressed significantly higher in LTS than STS. To identify exosomal miRs that could be transferred from ovarian cancer cells to modulate the TME, another miR sequencing analysis was performed on exosomes isolated from nine HGSC cell lines. It was found that miR-181c-3p was the only miR that present in exosomes out of those 50 miRs. Target prediction algorithms indicate that leukemia inhibitory factor (LIF) is a potential gene target of miR-181c-3p. Based on these findings, we hypothesize that miR-181c-3p can be transferred from ovarian cancer cells to various cell types within the TME to suppress LIF expression and increase CD8+ CTL activation and trafficking.

**Methods:** First, the expression of miR-181c-3p in FFPE tumor samples from LTS and STS was visualized using miRNAscope assay (Advanced Cell Diagnostics, Inc.). Next, correlation analysis was performed between miR-181c-3p expression and the intratumoral CD8+ CTL number in patient tumor samples. Lastly, the LIF mRNA and protein levels were evaluated in HGSC PEO1 and PEA1 cell lines transfected with control or miR-181c-3p inhibitor using qRT-PCR and Western Blot analyses, respectively.

**Results:** Tissue staining confirmed that miR-181c-3p is more prevalent in LTS than STS. Correlation analysis for miR-181c-3p expression and intratumoral CD8+ CTL count indicates a positive correlation. Significantly higher LIF mRNA and protein expressions resulted in cells transfected with miR-181c-3p inhibitor.

**Conclusion:** These results suggest that miR-181c-3p may increase CD8+ CTL activation and trafficking. QRT-PCR and Western blot analyses indicate that miR-181c-3p downregulates LIF expression.

**Keywords:** exosomal microRNA, ovarian cancer, miR-181c-3p, T-cells, leukemia inhibitory factor

University Outreach - Bryn Mawr College

Abstract Number: 134

### **Generating Artificial Antigen Presenting Cells for Adoptive T-Cell Therapy**

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**Background:** The transformation for cancers are commonly as a result of a series of hereditary and somatic DNA mutations that change healthy cells into cancerous ones. Therapeutic cancer vaccines have the potential to activate and strengthen the patients immune system against cancerous cells. For instance, the vaccination of patients with tumors can induce strong antigen specific anti-tumor T-cell responses that lead to cancer clearance. Reason development in the next generation sequencing of the human genome project paved the pathway to identify an alter gene/mutated genes. Using bioinformatics analysis, now it is possible to predict mutated peptides that can activate the immune system. The Major Histocompatibility complex (MHC) proteins, or the human Human Leukocyte Antigens (HLA) systems in humans, place an important role in presenting these mutated peptides to the immune system. We propose to develop a cell based tool for validating HLA binding patterns of the predicted neoantigens. We develop artificial presenting cells (APC's) by expressing HLA subtypes into HLA negative cell line K562. This will provide an opportunity to study: 1) Peptide and HLA specific interaction and 2) Specific T-cell interaction with cancer peptide.

**Methods:** With the use of bacteria transformation we are able to amplify our gene of interest using E. coli. The High Speed Plasmid Maxiprep Kit, provides us with a simple and fast silica membrane-based method to isolate high quality plasmid DNA from large recombinant E. coli culture. The ultrafast purification protocol, based on the remarkable selectivity of patented QIAGEN resin, allows the isolation of ultrapure, supercoiled plasmid DNA with high yields.

**Results:** After amplification and purifying HLA samples, HLA's were purified an Elution buffer. Elution buffer is used to wash away unwanted components from the solution, this is used to purify the final products. Using lower Elution volumes then the recommended 1000-1500 uL for the PureYield Maxiprep led to higher DNA concentrations, but lower overall yield. This can be beneficial when higher DNA concentration are preferred over higher yields for downstream applications such as transfection.

**Conclusion:** The data obtained suggested that high concentration of plasmid DNA were obtained. Almost all HLA samples resulted in a high concentration of plasmid DNA, which can be followed by transfection of K562 cells.

**Keywords:** Human Leukocyte Antigen (HLA), Antigen Presenting Cells (APC), peptides

University Outreach - University of Texas at Rio Grande Valley

Abstract Number: 135

**Impermissibility of Social Harm: Impersonal Harm, Social Values, and Genetic Enhancement**

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With the advent of CRISPR/Cas9 technology and the usage of preimplantation genetic diagnosis, the prospect to proactively prevent certain gene mutation-based disorders has become a real possibility. However, with therapeutic applications for genetic editing, also comes the ability for human beings to enhance their health beyond what was once thought capable, using genetic enhancement. One principle which seems to be critical to discussions of genetic enhancement is the theory of impersonal harm, which is harm done not to any individual, but rather to possible people through a set of decisions. Julian Savulescu and Guy Kahane argue that the principle of procreative beneficence can circumvent impersonal harm, and as such, would be an ideal principle by which to guide ethical genetic enhancement.

I will argue that the theory of impersonal harm has the capacity to fit a social aspect, and that because of this, the principle of procreative beneficence fails to be an ethical guideline. In replacement, I will further contend that the principle for the prevention of harm is able to account for social impersonal harm, and thus would be a better guideline for the ethical implementation of genetic enhancement. I will do this in a threefold manner: first, I will present and explain the concept of impersonal harm and show why it is a critical component of procreative genetic enhancement. From there, I will then recount Michael Sandel's social values argument, showing how these values are compatible with a notion of impersonal harm such that one must avoid altering these values in order to ethically implement genetic enhancement. Finally, I will present Savulescu and Kahane's argument for procreative beneficence and assert that due to the social implications of impersonal harm, that procreative beneficence cannot provide ethical guidance for enhancement, whereas an opposing principle, the principle of the prevention of harm, can prevent this impersonal-social harm.

Impersonal harm states that one can harm a potential world through a decision which creates a worse off world, so the absence of certain social values such as accepting the unknown would create a worse off world. Due to this, it seems that procreative beneficence selecting on a basis of "advantage" leads to a society in which certain traits are discriminated against in a eugenic fashion, whereas the principle of prevention of harm, which selects on a basis of "least suffering" would allow for diversity in traits, sustaining social values.

Refer to "results" section above

Keywords: Procreative Beneficence, Prevention of Harm, Impersonal Harm, Genetic Enhancement

University Outreach– Augustana College

Abstract Number: 136

### **Investigation of Phosphate Pro-Drug led Inhibition of ENO2 in ENO1-Deleted Cells**

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**Background:** Previous research has shown that by inhibiting the glycolic gene, ENO2, in ENO1-deleted tumor cells such as Glioblastoma results in the destruction of these cells. The most effective drug at targeting these cells is HEX, however it is unable to pass the cell membrane. Various side groups have been attached to HEX creating new compounds such as POMHEX in the hopes that they will solve this issue. Although POMHEX can pass the membrane, its large polar side chains cause it to be unstable in aqueous environments. The most promising compound is IBuVCY27, its cyclic benzyl sidechain makes it stable in aqueous environments while still being able to pass the cell membrane. With a few alterations, there may be a more effective form of IBuVCY27, which is the goal of this study.

**Methods:** By reacting 2-Hydroxy-5-methyl benzyl alcohol, 2-Hydroxy-5-methoxy benzyl alcohol, and 3,5-Di-tert-butyl-2-hydroxy benzyl alcohol with the shared intermediate of IBuVCY27, three new compounds, Drug 1, Drug 2, and Drug 3, were created. These new drugs, along with IBuVCY27 and HEX, were each tested in vitro on 96 well plates against non-ENO1 D423 cells and control groups of ENO1 D423 cells.

**Results:** The three drugs were properly created and confirmed using proton NMR. The results of the tests were quantified vs the normalized response of the drugs effect on ENO1 D423 control cells. IBuVCY27, Drug 1, and Drug 2 appear to be the best performing and shared with very similar results.

**Conclusion:** Since there are no definite conclusions that can be drawn, further testing to compare the stability and effectiveness in vivo on mice is needed to differentiate these three drugs. If one of them turns out to be successful, this could lead to a new form of treatment for these types of cancers.

**Keywords:** HEX, ENO1, ENO2, IBuVCY27, Pro-Drug

University Outreach– Augustana College

Abstract Number: 137

### **Clinical Implementation of DIGEST as an Evidence-Based Practice Tool to Grade Pharyngeal Dysphagia in Oncology: An Implementation Evaluation**

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**Background:** Pharyngeal dysphagia is a swallowing disorder commonly experienced in cancer patients as a result of tumor burden and radiation and/or surgery treatments. Pharyngeal dysphagia is characterized by swallowing difficulties involved in the transit of a bolus from the mouth to the esophagus that is visualized with the modified barium swallow study (MBS). This x-ray examination is performed by speech language pathologists (SLP) to visualize a patients' swallowing anatomy, physiology, and function. The DIGEST tool was designed to provide a reproducible method to grade results of the MBS in alignment with toxicity grading benchmarks of the Common Terminology Criteria for Adverse Events (CTCAE). One of the main goals of DIGEST was to allow scoring of swallowing severity in real-time clinical practice. The aim of this study was to evaluate the reporting rate of DIGEST in a comprehensive cancer center as a marker of feasibility of this tool in the clinical setting. Clinical implementation might improve interprofessional communication and increase dysphagic evidence-based practice (EBP).

**Methods:** An implementation evaluation was conducted in accordance with the STARI framework. All consecutive MBS conducted at MD Anderson Cancer Center between 2016-2021 were retrospectively reviewed. Flowsheet data and chart abstraction from the EPIC electronic health record (EHR) was conducted to identify presence of DIGEST grading in the MBS notes and to locate sources of missingness.

**Results:** Among 13,670 MBS conducted in 7,847 unique patients in the six-year period, 73% of MBS were conducted in males with a median age of 64 years, 62% among patients with head and neck cancer. The majority (90%) of MBS were conducted in outpatient encounters while 10% were conducted during inpatient admissions. DIGEST grades were reported in 93% of MBS records in the EHR (12,721/13,670 MBS, 95% CI: 1808.0-2426.0) with similar reporting rates over each study year. The most common reason for not reporting a DIGEST grade was due to the patient having a total laryngectomy (TL) (79.9%, 758/949), followed by leak/fistula (8.3%, 79/949), and MBS with severe bolus deviation (5.1%, 48/949).

**Conclusion:** : DIGEST is shown as a feasible EBP tool to grade pharyngeal swallowing function during an MBS in diverse oncology populations. Further research should be conducted to investigate the accuracy and effectiveness of DIGEST in a clinical environment. Most importantly, working to educate health professionals on the benefits of DIGEST implementation should be a top priority to improve patient's plan of care.

**Keywords:** Dysphagia, head and neck cancer, measurement, feasibility

University Outreach– Augustana College

### **CCR7 Immune Cell Receptor Expression in Inflammatory Breast Cancer**

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**Background:** Inflammatory breast cancer (IBC) is a rare form of breast cancer that is characterized by invasion of tumor emboli into lymphatic vessels. CCR7 is an immune cell receptor involved in immune cell movement into lymphatics that is upregulated in IBC. Our objective was to investigate the prevalence of CCR7 in estrogen receptor (ER) positive and negative IBC tissues to understand its role in IBC proliferation and potential novel therapies.

**Methods:** A human tissue microarray (TMA) of 36 primary breast tissue biopsies from IBC patients underwent immunohistochemical staining for CCR7. The TMA was converted to an e-slide and analyzed for CCR7 positivity using Aperio ImageScope software. Positivity percentage values reflected total CCR7 presence in both tumor and stroma. An expert pathologist reviewed the TMA to differentiate between positive tumor and stroma. Samples containing tumor (24 out of 36) were evaluated for CCR7 positivity, staining pattern, percent tumor stained, and intensity. Results were interpreted alongside ER status.

**Results:** All samples expressed CCR7 positivity in either stromal or tumor cells (Median positivity = 44%). Of the 24 samples containing tumor, 23 (96%) had CCR7-positive tumor. 65% of positive samples had complete membranous pattern, 91% had 3+ intensity, and 61 had 100% tumor stain. Of the CCR7-positive samples with known ER data, 9 were ER positive and 8 were ER negative.

**Conclusion:** Since CCR7 was highly expressed in 96% of tumor-containing samples across ER+ and ER- subtypes, it can be investigated as a target for future personalized IBC treatments. Because all samples lacking tumors still exhibited stromal CCR7 positivity, the effect of stromal CCR7 on IBC development is an additional point of future investigation. Further analysis includes obtaining ER and HER2 data for samples of unknown status to further assess the relationship between CCR7 expression and various receptors implicated in IBC. Additional studies might include patients for whom we have medical images, as CCR7 may be involved in differences in IBC clinical presentation.

**Keywords:** IBC, CCR7, estrogen receptors

University Outreach– Augustana College

**An examination of patients' and providers' communication about de-escalating testing for prostate cancer.**

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**Background:** De-escalating surveillance testing by dropping biopsies or spacing out testing for prostate cancer progression will personalize care and optimize treatment outcomes. However, the lack of communication and educational information about prostate cancer diminishes the quality of cancer care, which could result in overtreatment and over-testing for low-risk prostate cancer patients.

**Methods:** This was a cross-sectional study recruiting patients from the MD Anderson Cancer Center and clinicians in the U.S. Eligible patients were men with localized prostate cancer, on active surveillance for at least 1 year,  $\geq 65$  years of age, had no evidence of disease progression, and spoke English. Eligible clinicians were  $\geq 18$  years of age, spoke English, and worked with patients with prostate cancer. After-consent patients and clinicians completed a survey about clinicians' approaches and patients' understanding of what is involved in low-risk prostate cancer. Means, medians, proportions, and ranges for the survey items were calculated. Stata was used for analysis.

**Results:** 36 patients (Age range: 65-82 years, = 71.36, SD = 5.13; African American (AA): 5.56%, White: 94.44%) were recruited from the MD Anderson Cancer Center. 26 clinicians (10 female; age range: 27-64 years, = 44.65, SD = 9.61; years of practice range [1.5,30], SD= 9.31; Black or AA: 11.54%, Asian: 19.23%, White: 69.23%) were recruited in the U.S. Patients and clinicians agreed on effective communication about the fact that patients had low-risk prostate cancer and they were unlikely to die from it. Most clinicians (84.62%) communicated that those patients would have testing for their prostate cancer, and 56% of clinicians told patients their prostate cancer did not need any treatment at this time. However, patients still had an unclear picture of what the future may hold for their treatment. Some clinicians never (7.69%) or rarely (34.62%) handed out educational materials to patients, which shows the inconsistency in informative communications about prostate cancer between clinicians and patients.

**Conclusion:** Patients need to get educational materials about prostate cancer at the time of their diagnosis or before de-escalating surveillance testing. Communication interventions help patients decide when de-escalating surveillance testing is appropriate, which supports patients to make well-informed decisions and provides them with better healthcare services that align with their values.

**Keywords:** prostate cancer, cancer communication, de-escalation, shared decision-making, patient-provider communication.

University Outreach– Augustana College



Abstract Number: 140

**Investigating a Potential Relationship Between Distinct Cancer-Associated *Lactobacillus iners* and Chemoradiotherapy Resistance in Cervical Cancer Patients**

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**Background:** Chemoradiotherapy (CRT) is the combined efficacious administration of chemotherapy and chemoradiation as an antineoplastic approach. However, instances of poor clinical response to treatment, diminished recurrence-free survival rates, and incurable relapse in cervical cancer patients are alarming, prompting the investigation of potential markers of CRT resistance. *Lactobacillus iners* do not exhibit protective capabilities in the cervical microbiome as this species cannot maintain intravaginal acidity and contributes to the onset and progression of infectious conditions. Previous research suggests that tumor microbiomes predominated by *Lactobacillus iners* are significantly associated with poor patient outcomes, unfeasible recoveries, and dysfunctional immune responses. There is an insufficient amount of understood and certified molecular markers to pinpoint patients who will respond poorly to treatment. To remedy this, the tumor microbiome in cervical cancer patients serves as a manifestation of the radio-sensitivity of cervical cancer cells, which can be thoroughly examined to detect how these cells will react to CRT interventions.

**Methods:** The cervical cancer cell line, HeLa cells, were co-cultured with supernatants from the ATCC 55195 *L. iners* strain, *L. iners* derived from the cervical swabs of patient 366, and *L. iners* isolated from the cervical swabs of patient 370. These treatment groups were evaluated and compared to negative controls of NYC III and MEM 1X mediums. Cisplatin chemotherapy was administered at doses of 0.5 – 1.0 µg/mL concurrent with 1.5 – 3.0 Gy of chemoradiation. Clonogenic cell survival assays were conducted through crystal violet staining in conjunction with Cell Titer-Glo Luminescent cell viability assays.

**Results:** It is expected that there will be a strong association between tumors subjugated by *Lactobacillus iners* and poor clinical response to CRT, which is emblematic of curtailed recurrence-free survival rates and an impaired quality of life in cervical cancer patients. Numerous procedures were performed prior to the supernatant experiment in order to ascertain baseline control groups while establishing validated responses of cervical cancer cells to chemoradiotherapy before co-culturing the cells with *Lactobacillus iners*.

**Conclusion:** This detrimental in-vitro effect of *Lactobacillus iners*' supernatants on cervical cancer cells must be further scrutinized to substantiate it as a novel marker in the tumor microbiome that predicts poor clinical responses to chemoradiotherapy modalities. The Colbert Laboratory has discerned that the pathotype of cancer-associated *Lactobacillus iners* promote in-vitro resistance of cervical cancer cells while modifying the local tumor immunologic microenvironment. Bearing this in mind, future objectives to enhance the curative properties of CRT must include the generation of low-risk, focused interventions that transform the cervical microbiome while mitigating the dominance of *L. iners* through the proposed employment of bacteriocins, lytic phages, or probiotics.

**Keywords:** *Lactobacillus iners*, Chemoradiotherapy, Cervical Cancer, Microbiome

University Outreach– Baylor University

**STING Activation Combined with cMET Inhibition and ICB Therapy Promotes Anti-Tumor Effect in DEN Mouse Model**

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Background: Hepatocellular Carcinoma (HCC) accounts for 90% of all liver cancers. Surgical resection, radiotherapy, liver transplantation, and Immune Checkpoint Blockade (ICB) are viable options; however, patient prognoses remain poor.

Targeting aberrant Mesenchymal-to-Epithelial Transition (MET) is a possible clinical treatment. Unfortunately, inhibition of MET upregulates PD-L1 (Programmed Cell Death Ligand) expression which triggers immunosuppression. STING agonists, yet another therapeutic, promote tumor-specific T-cell responses by stimulating Interferon- $\alpha$  (IFN $\alpha$ ) production.

The purpose of our experiment was to investigate the efficacy of a Triad Therapy joining cMET inhibition,  $\alpha$ PD-1, and STING agonists. We hypothesized that the immunosuppressive effect of cMET inhibition would be counteracted through combination therapy with  $\alpha$ PD-1 and increase tumor-infiltration of lymphocytes.

Methods: To establish that cMET inhibition upregulates PD-L1 expression, we plated 4 6-well plates ( $2.5 \times 10^5$  cells/well) for each of 3 human cell lines (SNU-449, C3A, PLC/PRF/5) and 1 mouse cell line (HCA-1). Each of three different cMET inhibitors were given to each cell line at 10  $\mu$ M and 1  $\mu$ M, and PD-L1 expression was measured through flow cytometry. Capmatinib was chosen for our in-vivo experiment due to lowest levels of PD-L1 expression.

The *in vivo* experiment began with implanting chunk tumors subcutaneously in the right flanks of 40 HCC-resistant DEN mice randomized into 8 groups. Once tumor burden exceeded 50mm<sup>3</sup>, the 10-day treatment cycle began. Capmatinib (10 mg/kg) was delivered daily via gavage. The  $\alpha$ -PD-1 antibody (250  $\mu$ g per mice) was delivered intraperitoneally every other day, and the STING agonist (10  $\mu$ g/mice) was administered intratumorally thrice. Ultimately, mice were euthanized, and their tumors were abstracted and analyzed using flow cytometry for tumor-infiltrating lymphocytes.

Results: We found that Triad Therapy led to statistically significant antitumor effects in the mice. Primarily, relative to other treatment combinations, Triad Therapy increased the tumor-infiltration of CD4<sup>+</sup> lymphocytes. The triple-therapy combination also enhanced CD8<sup>+</sup> T<sub>eff</sub> and NK cells infiltration of the tumor microenvironment (TME). Additionally, Triad Therapy decreased the presence of CD4<sup>+</sup>FoxP3<sup>+</sup> T<sub>reg</sub> cells in the TME which suppress the activated immune response.

Conclusion: The goal of our study was to explore the potential for combination therapy to increase lymphocyte infiltration of the tumor microenvironment. Our results confirm that combining cMET inhibition, STING agonist, and anti-PD-L1 therapies is a promising avenue for further research. Our study was limited by the number of mice in our *in vivo* experiment; still provides basis for future experiments that are scaled proportionately to include more samples.

Keywords: Immunotherapy, Anti-PD-1, cMET Inhibition, Hepatocellular Carcinoma.

University Outreach– Baylor University

Abstract Number: 142

### **Manipulation of Abro1 Localization in U2OS Cells**

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**Background:** When a DNA replication fork stalls, it can lead to replication stress. Defects in stalled fork protection in the replication stress response leads to genomic instability. Abro1 has been found to be a key factor in protecting the integrity of stalled replication forks. Abro1 is present in both the cytoplasm and nucleus of the cells. The functional importance of the localization of Abro1 is not clear. To further study its localization, we generated two mutants of Abro1 with either a Nuclear Localization Signal (NLS) or Nuclear Export Signal (NES), which only localizes the Abro1 to either the nucleus or cytoplasm.

**Methods:** Abro1-NLS and Abro1-NES were generated on pENTR-Abro1 by mutagenesis PCR to generate the mutant genes. The PCR products were transfected to E. Coli, which was then spread on Luria Broth (LB) plates and screened. Individual clones were picked and sequence verified. The products were then transferred from pENTR vectors to destination vectors to generate HA-Abro1, HA-Abro1-NLS, and HA-Abro1-NES. These recombination products were then transfected to E. Coli and individual clones with accurate recombination were identified. The plasmids were then extracted and used for transient expression in U2OS cells for 48 hours. A western blot was performed to verify expression. Immunofluorescence was performed and images were taken by a Confocal microscope.

**Results:** Western blot results indicated that the Abro1-NLS and Abro1-NES mutants were successfully generated with the correct localization signals. The immunofluorescence results showed that the NLS signal successfully localized Abro1 inside of the nucleus and the NES signal successfully localized Abro1 in the cytosol, outside of the nucleus.

**Conclusion:** The successful generation of the two Abro1 mutants is an important first step in the understanding of Abro1. In the future, these mutants could be used for further testing of the different functions of Abro1 based on its localization and how these functions interact. Thus, with a deeper understanding of Abro1, we can have an increased understanding of genome stability which can have implications for tumor suppression.

**Keywords:** Abro1, Stalled Replication Fork Stability

University Outreach– Baylor University

Abstract Number: 143

### **Ki67 Quantitation in Breast Cancer: A Comparative Analysis of Four Counting Methodologies**

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**Background:** Ki67, an important tumor marker in breast cancer, is associated with cell proliferation. Ki67 is suggested to be of clinical value in determining whether adjuvant chemotherapy is necessary in ER positive/HER2 negative breast cancer patients. While immunohistochemical stains are commonly used to assess Ki67, quantitation is limited by lack of standard scoring methods. Most laboratories visually or digitally quantify Ki67 in the entire tumor. Another method is quantifying Ki67 in “hotspots” with the highest nuclear labeling. Recently, the International Ki67 Working Group (IKWG) proposed quantifying areas of varying Ki67 positivity. Currently, no single method has gained universal acceptance as a reliable method for Ki67 quantitation. Therefore, the goal of this study was to identify a reliable method of Ki67 quantitation by comparing four methodologies: Visual Total Count (TC), Digital Total Count (TC), Hotspot Count and IKWG Unweighted (IKWG-U).

**Methods:** Ki67 immunohistochemical stains of 100 cases of HER2 negative/ER positive breast cancers were scored and categorized into low (<17%), moderate (17-35%) and high Ki67 (>35%). Visual TC method, the method used in our institution, was the consensus score for Ki67 staining in the entire invasive tumor and served as the standard. Digital TC method quantified Ki67 in the entire tumor by digital analysis (Image J). IKWG-U method quantified Ki67 in four areas of different staining densities. Hotspot Count quantified Ki67 in representative areas with the highest Ki67 labelling. The time required for each method was noted.

**Results:** In comparison to Visual TC, the Digital TC and IKWG-U methods each altered Ki67 categories in 3 of 100 cases. Hotspot Count altered Ki67 categories in 16 of 100 cases. The scores from each method were compared to Visual TC using a scatter plot and linear regression, yielding R<sup>2</sup> values of 0.9756, 0.9776, and 0.8678 for Digital TC, IKWG-U and Hotspot Count respectively. Average Time required for Visual TC was < 2 mins, Digital TC was 28 mins, IKWG-U was 10 mins and Hotspot Count was 6 mins.

**Conclusion:** This study demonstrated high correlation amongst Visual TC, Digital TC and IKWG-U methods for Ki67 evaluation. While these methods yielded comparable results, Visual TC required the least amount of time and can be easily utilized in a clinical setting. These results validate the use of Visual TC as a viable and effective method to quantify Ki67 in our clinical work. Further studies to establish clinical relevance of these quantitation methods and their impact on clinical management is necessary.

**Keywords:** Ki67, breast, pathology, quantitation

University Outreach– Baylor University

Abstract Number: 144

**Role of Antimicrobial Peptide, SAA3, in inducible protection by Pam2-ODN in mouse lung epithelial cells**

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**Background:** Pneumonia is an invasive disease that results in the inflammation of the alveolar sacs in the lungs, causing fluid retention. The retention of fluid results in the impairment of breathing and can cause sepsis, often leading to death. The disease is most commonly seen in those with a weakened immune system, such as those undergoing chemotherapy treatment. Previous studies have indicated that the epithelial cells of the lungs can be stimulated to provide protection against these pneumonias. This stimulation was induced by treatment consisting of the combination of two TLR agonists. These agonists, TLR 2/6 and TLR9 work in synergy by recognizing the motifs Pam2CSK (Pam2) and oligonucleotide (ODN) M362, respectively, triggering a signaling cascade which modulates the immune response after pathogenic infection. Combined, the TLR's are recognized as Pam2-ODN. Previous microarray analyses have indicated upregulated genes coding for antimicrobial peptides (AMPs) after the addition of PAM2-ODN treatment. One of them, Serum amyloid A (SAA), is an AMP that is known to modulate inflammatory feedback responses. A murine isoform of the protein, SAA3, produces similar responses in epithelial cells. This response renders the protein as a biomarker for infection, allowing us to use it as a measurement tool following the administration of treatment. As such, this study aimed to validate the microarray analysis by performing qRT-PCR ran at various time points to better understand the gene expression level over time.

**Methods:** This study consisted of various timed trials ran in five phases: cell culture, addition of treatment, RNA extraction, cDNA conversion, and qRT-PCR analysis. Once the cells reached full confluence, treatments were added according to their designated time frame. Once the time was complete, cells were then lysed and RNA was extracted using a spin column. Gene expression of SAA3 was measured by qRT-PCR relative to housekeeping gene 18s. The data were analyzed using the GraphPad software.

**Results:** Data showed increased levels of SAA3 production in treatment group, at the 2 and 4 hour time frames.

**Conclusion:** The results show that SAA3 is expressed significantly at 2 hours and 4 hours due to Pam2-ODN treatment. Pam2-ODN induces a protective response through AMPs such as SAA3, especially at these time frames in the MLE15 cell line. Further studies involving in-vitro infection model must be performed to assess the protectiveness of SAA3 and other AMPs induced by Pam2-ODN to gain more insight into the mechanism of action behind this protection.

**Keywords:** Antimicrobial Peptide, lung epithelium, synergy, inducible protection

University Outreach– Nova Southeastern University

Abstract Number: 145

**Using the Persuasive Design Model to Refine a Novel Stimuli-Responsive Polymeric Sensor in Head and Neck Cancer Patients**

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**Background:** We conducted two user-centered tests with head and neck survivors to evaluate long-term usability of a novel sensor to detect developing dysphagia after radiation. Radiation-associated dysphagia is permanent and occurs in 40% of laryngeal/pharyngeal cancer patients treated with curative radiation. We prototyped a stretchable neck-worn epidermal dual-layer strain/sEMG sensor that measures swallowing muscle activity to aid in the earlier detection of dysphagia development. Ideally, home-based monitoring with the sensor during the 1-2 year post-radiation treatment period would detect developing dysphagia in time to initiate preventive interventions. However, most U.S. patients abandon wearable health technologies within a few months, lessening their clinical impact. To sustain patient engagement, user-centered testing is needed but often neglected in the development of these technologies. To evaluate the sensor's long-term usability, we used the persuasive design model to test four parameters of technology: ease of use, user feedback, credibility and social support.

**Methods:** Two studies were conducted to gauge patient preferences. First, we asked 138 pharyngeal/laryngeal cancer survivors who were 2-5 years post-radiation: a) their understanding of the sensor's rationale, b) willingness to wear the sensor for 1 year post-radiation and c) design preferences. Second, we conducted swallowing tests with 27 laryngeal/pharyngeal cancer patients while wearing the sensor prototype and repeated the questions from Study 1.

**Results:** Study 1: N=138 survivors answered questions about future trial participation in a 9-month long sensor study: 83.5% (n=116) of the participants indicated willingness to participate. Of those unwilling to participate (16.5%, n=23), 9 months was perceived as too long of a trial period (86.4%, n=19). Study 2: After swallowing tests with the sensor, 77.8% (n=21) indicated willingness to participate, citing support for the preventive rationale of the epidermal sensor as the main reason for participation (100%, n=21). Those unwilling to participate (22.2%, n=6) also indicated 9 months too long of a trial period (83.3%, n=5). Patients communicated that sensor application was the main barrier to sustained use while conveying the need for instant haptic feedback. Participants also expressed that sending sensor data to doctors would be a more efficient way of monitoring dysphagia development.

**Conclusion:** In the framework of the persuasive design model, these results indicate strong support for further development of the sensor's ease of use, user feedback and credibility. Survivors are willing to use home-based technologies to support adherence to preventive strategies, but this willingness is dependent on the sensor being compatible with their lifestyle and post-treatment radiation side effects.

Keywords: Head and Neck Cancer, Sensor, Persuasive Design, Radiation-associated dysphagia, Wearable health technology

University Outreach– University of Notre Dame



### **Targeting DNA damage repair pathways in diffuse intrinsic pontine gliomas**

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**Background:** Diffuse intrinsic pontine gliomas (DIPGs) are a uniformly fatal class of highly aggressive pediatric brainstem tumors. In the absence of effective treatments and more than 250 failed clinical trials, prognosis remains exceptionally poor at a median survival of 9-11 months post-diagnosis. Data from our lab has shown that DNA damage machinery genes, downstream of BRAC1 and PARP1, are upregulated in DIPG tumors. Their expression positively correlated with that of the oncogene, REST. **Objectives:** To explore the therapeutic potential of targeting BRAC1 and PARP1 signaling using drugs that can target PARP and RAD51 proteins. We hypothesize that REST elevation in DIPGs induces DNA double stranded breaks and these cells are dependent on repair for their survival. Therefore, blocking this repair machinery will induce cellular stress and cell death.

**Methods:** DIPG cell lines (3 parental and 3 isogenic REST overexpressing) were exposed to the PARP inhibitor - Talazoparib and the RAD51 inhibitor – Berberine at various concentrations for 5 days. Drug sensitivity was evaluated by determining cell viability using an MTT assay. Flow cytometry was used to conduct cell cycle analysis.

**Results:** Talazoparib increased cell death significantly in multiple cell lines at various concentrations. Flow cytometry revealed a G2/M-phase cell cycle arrest at a concentration of 1600nM Talazoparib. Berberine showed a small decrease in cell growth in DIPG cells but induced G1 arrest at a drug concentration of 2000nM. Cell lines with high-REST expression demonstrated greater sensitivity to both drugs. Phase contrast imaging revealed a decline in neurosphere formation following drug treatment.

**Conclusion:** PARP and RAD51 show promise as potential therapeutic targets for DIPG. High-REST expression further sensitizes tumors to drug treatment. Decrease in neurosphere formation suggests a decline in the stem/progenitor cancer cell population.

**Keywords:** Diffuse intrinsic pontine glioma, DNA damage repair, cytotoxicity, cell-cycle arrest, REST

University Outreach– University of Notre Dame

Abstract Number: 147

**Combined inhibition of IRS-1 and HDAC blocks uveal melanoma cell survival and induces apoptosis.**

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**Background:** Uveal Melanoma (UM) is a rare subtype of melanoma of the uveal tract of the eye. The primary tumor is treatable, but poor prognosis comes from metastatic disease to the liver. There is an urgent need to develop new therapies as only one FDA approved therapy exists. IRS-1 (insulin receptor substrate-1) inhibition partially blocked UM cell survival and cell migration. Single agent therapies in melanoma lack effectiveness in the long run. To develop a combination therapy, a high throughput combination drug screen with IRS-1 inhibition was performed and showed a possible synergistic effect with the histone deacetylase (HDAC) inhibitor, Belinostat (Bel), in UM cell killing activity. To validate these findings, I performed combination treatments with NT157 (IRS-1 inhibitor) and Belinostat on UM cell lines to determine their combined effect on UM cell survival and the mechanism of such.

**Methods:** We used UM cell lines: MM28, MP38, 92.1, MP41, MP65, MEL202, and MEL20-06-039 with representative genotypes. NT157 and Belinostat were purchased from SelleckChem. We assayed UM cell survival using MTT assays and a clonogenic colony formation assay. Routine western blots were used for the molecular analysis of protein markers.

**Results:** High Throughput Drug Screen (HTS) for novel combination therapy strategies for UM: Our initial combination HTS allowed us to predict a synergistic relationship between IRS-1 and HDAC inhibition. Over 2000 approved drugs were tested for UM cell killing activity and filtered down to 31 candidates for a combination screen. From this Belinostat was chosen as it showed a good synergy score with NT157 in combination. UM cell survival is efficiently blocked with combined treatment of Belinostat and NT157: Combination treatment with NT157 and Belinostat substantially improved inhibition of UM cell survival over individual single agent treatments from our MTT assays, which we confirmed using clonogenic colony formation assays with UM cells. Apoptosis was induced in UM cells treated with NT 157 and Belinostat in combination: Western blots indicated that single agent treatments targeted respective pathways and combination treatments induced apoptosis (increased PARP breakdown) in UM cells.

**Conclusion:**

- IRS-1 inhibition partially blocked UM cell survival and migration.
- Unbiased high throughput drug screen indicated synergy between IRS-1 and HDAC inhibition.
- This was validated in in vitro assays in the current study with nanomolar doses of each targeting agent.
- Dual targeting of IRS-1 and the HDAC pathways improved UM cell killing over single agent treatments.
- Apoptosis was extensively induced in UM cells with combination treatments.

**Keywords:** Uveal Melanoma, liver metastasis, IRS-1, HDAC, apoptosis

University Outreach– University of Notre Dame

Abstract Number: 148

### **Specificity of the S9.6 Antibody in the Detection of R-loops and RNaseH1**

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**Background:** Previous studies involving the specificity of the S9.6 antibody in the detection of R-loops rely primarily on immunodetection which specifically binds DNA-RNA hybrids. Regulation of these R-loops via RNaseH1, a protein that cleaves R-loops, is necessary to maintain genomic stability. Studies investigating the underlying molecular basis and mechanisms of the specificity of the S9.6 antibody in the detection of R-loops and the RNaseH1 protein are still not completely understood, especially not in cholangiocarcinoma. To better understand these underlying mechanisms, a study would need to be conducted that establishes that R-loops can accurately be detected using immunofluorescent analysis using the S9.6 antibody and that it is specific to RNaseH1 in SSP25 cells.

**Methods:** We conducted an immunofluorescence microscopic analysis using the S9.6 antibody and DAPI to image DNA signals for R-loops and stain nuclei of the SSP25 cell line with different shPTEN samples and ATR inhibitors. Using CalFectin, we transfected RNaseH1 DNA into 293T cells. We then ran a western blot to test the RNaseH1 WT and its mutants (WKKD and D210N) to test the specificity of RNaseH1 and its expression with respect to the S9.6 antibody. The last step in the methods section would have been transfecting RNaseH1 DNA into the SSP25 cell line, running an identical western blot, and then another immunofluorescence analysis.

**Results:** Immunofluorescent analysis shows that the S9.6 antibody can accurately detect S9.6 signals. The analysis also shows that there was a decrease in S9.6 signal intensity in the ATRi BAY1895344 with both PTEN knockdown samples. Immunofluorescent analysis also showed that there is an increase in S9.6 signaling intensity in both shPTEN samples. Western blot analysis shows the expression of RNaseH1 in all RNaseH1 samples except for the one containing the GFP plasmid. RNaseH1 diminished the expression of GFP in PeGFP-RNaseH1. V5 resulted in the overexpression of RNaseH1.

**Conclusion:** Immunofluorescent analysis revealed the S9.6 antibody's ability to accurately detect S9.6 signals and stain nuclei. Immunofluorescent analysis also showed that ATRi BAY1895344 decreases the nuclear intensity of the S9.6 signal in shPTEN and that shPTEN generally increases the nuclear intensity of the S9.6 signal. The western blot showed the expression of RNaseH1 was detected in all RNaseH1 mutants and in the WT except for the one containing the GFP plasmid. Future studies will need to test the expression of RNaseH1 in SSP25 cells and the detection of R-loops.

**Keywords:** S9.6, RNaseH1, R-loops, Cholangiocarcinoma, ATRi

University Outreach– University of Notre Dame

**Bismuth Nanoparticle and Dipyridamole-loaded Electrospun Polymeric Scaffold as Radiopaque Bioresorbable Drug-Eluting Vascular Graft**

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**Background:** Arteriovenous grafts are used as an interventional access point for patients on dialysis. However, upon failure, graft placement can lead to neointimal hyperplasia (NIH). Anti-platelet and vasodilator drugs such as dipyridamole (DPA) mitigate NIH and long-term patency post graft placement. In addition, nanoparticles allow for visualization and long-term monitoring of these absorbable medical devices. This study aims to develop a bismuth nanoparticle (BiNP) and DPA-loaded scaffold made of polycaprolactone (PCL) and polyethylene glycol (PEG). These scaffolds were tested for their efficacy as novel bioresorbable, radiopaque, drug-eluting vascular grafts.

**Methods:** BiNPs were synthesized via the thermal decomposition method and its size was determined by transmission electron microscopy (TEM). Solutions of PCL (80,000kDa), PEG (8,000kDa), BiNP, and DPA were electrospun into 3cm scaffolds, and physiochemical properties were characterized. Scaffolds were monitored over 6 weeks in terms of drug (UV-vis absorption) and nanoparticle (elemental analysis) released, tensile strength (MTESTQuattro universal testing system), and radiopacity (Bruker micro-computed tomography). Immortalized human vascular endothelial cells (EC-RF24) and vascular smooth muscle cells (MOVAS) were used to determine the scaffolds cytotoxicity using alamarBlue assay. Grafts were surgically implanted in rats to begin in vivo imaging and efficacy studies.

**Results:** BiNPs size was  $3.44 \text{ nm} \pm 0.59 \text{ nm}$  as determined by TEM. Morphology and physiochemical properties of the scaffolds varied. Fiber diameter increased with the addition of BiNP and DPA (PCL-PEG-BiNP-DPA:  $2.53 \pm 0.64 \text{ } \mu\text{m}$  vs. PCL-PEG:  $1.56 \pm 0.59 \text{ } \mu\text{m}$ ). Mechanical strength decreased with the addition of BiNP (PCL-PEG:  $6.28 \pm 2.77 \text{ MPa}$  vs. PCL-PEG-BiNP:  $2.12 \pm 0.42 \text{ MPa}$ ). DPA-loaded grafts released ~50% of the drug over 7 days (PCL-PEG-DPA:  $49.62\% \pm 0.7\%$  vs PCL-PEG-BiNP-DPA:  $45.85\% \pm 3.7\%$ ), which increased to ~60% release over six weeks (PCL-PEG-DPA:  $60.50\% \pm 2.5\%$  vs PCL-PEG-BiNP-DPA:  $55.90\% \pm 4.9\%$ ). BiNP-loaded grafts released between 1-2% of the nanoparticle in the first week, which correlated with the quantification of radiopacity in Hounsfield units (HU, PCL-PEG-BiNP-DPA:  $1001.2 \pm 111.8 \text{ HU}$  at week 0 vs. PCL-PEG:  $-880.4 \pm 13.6 \text{ HU}$ ). EC-RF24 and MOVAS remained viable in the presence of BiNP and DPA treated media. The presence of DPA in grafts increased blood lysis by about 2% (PCL-PEG-DPA:  $1.86\% \pm 1.1\%$  vs PCL-PEG-BiNP-DPA:  $2.54\% \pm 0.5\%$ ).

**Conclusion:** Trilayer scaffolds made of PCL and PEG and loaded with both DPA and BiNP demonstrated increased radiopacity with no detrimental effects on epithelial or vascular smooth muscle cells, and an effective release of dipyridamole over time. These results confer its advantage for serial non-invasive imaging over time to assess degree of polymer resorption and potential for in vivo NIH inhibition.

**Keywords:** Bismuth, radiopacity, medical device

University Outreach– University of Notre Dame

## UPWARDS Summer Program

Abstract Number: 150

### **UBA1 Depletion Sensitizes Ovarian, Breast, and Colorectal Cancer Cell Lines to Olaparib PARP inhibitor**

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**Background:** Protein poly ADP-ribosylation (PARylation) is a post translational modification which is catalyzed by poly (ADP-ribose) polymerases (PARPs). This post translational modification regulates several biological processes such as DNA damage response, apoptosis, chromatin remodeling, mitosis and transcriptional regulation. Specifically, the catalytic activity of PARP proteins is important for single stranded break repair (base excision repair). Depletion or inhibition of PARP catalytic activity converts ssDNA lesions to DNA double stranded breaks which are repaired by homologous recombination repair (HR). In recent years, PARP inhibitors have been established as a proficient strategy to treat HR deficient cancers. But it is less efficient to treat homologous recombination efficient and RAS mutant tumors. Here we have attempted to identify candidates whose loss of function can make homologous recombination efficient and RAS mutant tumors more sensitive to PARP inhibitors.

**Methods:** We performed genome wide CRISPR KO screens in ovarian and breast cancer cell lines to identify target genes whose loss can make cells more sensitive to PARP inhibitor treatment. Further, we validated the CRISPR KO screen findings by using cell proliferation and clonogenic potential of the cancerous cells.

**Results:** We analyzed the screen by using MaGeCK and DrugZ pipelines. We identified several key components of the DNA damage repair pathway such as BRCA1, BRCA2, RAD50, FANCD2 and FEN1. In addition to these known players in the DNA damage repair pathway we found UBA1 (a major ubiquitination activation enzyme) as a top depleted gene. To investigate the effect of UBA1 depletion on Olaparib sensitivity, We Knockdown UBA1 in ovarian, breast and colorectal cancer cell lines using siRNAs targeting UBA1. We confirmed the UBA1 knockdown efficiency at protein level by using western blot. We found UBA1 depletion results in a significant increase in Olaparib sensitivity, compared to the control siRNA treatment in all tested cell lines. We also observed a very strong reduction in colony forming potential in UBA1 depleted cells treated with different doses of Olaparib in comparison to control siRNA cells treated with different doses of Olaparib. Together, these findings indicate that the UBA1 depletion sensitizes ovarian, breast and colorectal cancer cells to Olaparib treatment.

**Conclusion:** We Identified UBA1 as top depleted gene in our genome wide CRISPR KO screens which suggests that UBA1 loss of function sensitizes the ovarian and colorectal cancer cells to PARPi. Validation experiments with siUBA1 further confirms our genome wide CRISPR KO screen findings.

**Keywords:** Olaparib PARP inhibitor treatment, UBA1, genome-wide CRISPR knockout

UPWARDS Summer Program

Abstract Number: 151

### **Biomechanical Processes of the Mullerian Duct During Sex Development of Mice**

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**Background:** Amniotes, regardless of sex, develop bipotential gonads, Mullerian Ducts (MD), and Wolffian Ducts (WD). In mice the MD is fully formed by embryonic day 13.5 (E13.5). The MD is the precursor to the female reproductive system and will develop into the oviduct, uterus, cervix, and upper vagina. The WD is the precursor to the male reproductive system and will develop into the epididymis, vas deferens, and seminal vesicles. In males, the bipotential gonads develop as testes and release anti-Mullerian hormone (AMH) which will eliminate the MD. Additionally, the testes release testosterone which stabilizes the WD. Without AMH or testosterone in females, the MD will remain and the WD will passively degrade.

**Methods:** To visualize these processes in the mouse reproductive tracts three methods were used: X-gal staining of Lim1-LacZ (a marker of MD and WD epithelial cells) mice, Ex Vivo Organ Culture and Whole Mount immunofluorescence. Reproductive tracts from Lim1-LacZ positive mice were dissected, stained with X-gal, and cleared with BABB to visualize the MD and WD. The Ex Vivo Organ Culture used a glass bottom disk with a filter and growth medium to support the tissue. The reproductive tracts were imaged on the dissecting and spinning disk microscopes. Whole mounts were stained with Keratin 8 and Cleaved Caspase 3, mounted on slides, and imaged on the confocal microscope.

**Results:** The X-gal staining showed that fusion of the MD occurs in males and females at E14.5, and there is regression in the MD of males at E14.5. Ex Vivo Organ Cultures showed fusion at E14.5 and the elimination of the MD in males by E18.5. The Whole Mounts showed apoptosis in throughout the MD in males starting at E.14.5.

**Conclusion:** Fusion is a process that begins at E14.5. After E14.5 in males, the MD begins to regress in three stages before it is eliminated: thinning, breaking, and contracting. Apoptosis is seen in the MD of male mice starting at E14.5 and helps to aid the process of regression. Observed cellular behaviors and movements of the MD, observed by static and time-lapse imaging, during MD fusion and regression also suggest biomechanical processes are involved.

**Keywords:** Sex, Biomechanics, Sex Differentiation, Mullerian Duct, Microscopy

UPWARDS Summer Program

Abstract Number: 152

**Inhibition of toll-like receptor 4 and STAT3 changes cell proportions in livers affected by nonalcoholic steatohepatitis.**

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**Background:** TAK-242 is an inhibitor of Toll-like receptor 4 (TLR4), which is a known driver of hepatocellular carcinoma (HCC) development in non-alcoholic steatohepatitis (NASH)-associated HCC, as identified in the HepPten- mouse model by the Beretta lab. Signal Transducer and Activator of Transcription 3 (STAT3) is known to contribute to liver fibrosis and HCC in mice as also shown by the Beretta lab. This experiment was designed to determine whether TAK-242 and TTI-101, an inhibitor of STAT3, change the liver cell proportions to prevent HCC.

**Methods:** CIBERSORTx was used to generate a cell deconvolution analysis using a landscape adult liver mouse matrix from Nanostring and RNA sequencing data from both the HepPten- NASH mouse livers treated by the STAT3 inhibitor and treated by the TAK-242 inhibitor. To correlate this data's results in human livers, another cell deconvolution analysis was run on data collected by The Cancer Genome Atlas from 371 HCC tumors and paired normal adjacent samples using the landscape adult liver mouse matrix, a SafeTME matrix, and an adult immune census matrix.

**Results:** Siglech-high dendritic cells showed a statistically significant decrease when both TLR4 ( $p=0.0138$ ) and STAT3 ( $p=0.0004$ ) were inhibited. Endothelial cells ( $p=0.0107$ ) and mt-nd4-high hepatocytes ( $p=0.0163$ ) showed a statistically decrease in cell proportions when TLR4 (was inhibited and a decreasing trend when STAT3 was inhibited. Spp1-high epithelial cells ( $p=0.0163$ ), Kupffer cells ( $p=0.0003$ ), and pericentral hepatocytes ( $p=0.0005$ ) showed a significant decrease in cell proportions when STAT3 was inhibited, and a decreasing trend when TLR4 was inhibited in epithelial and Kupffer cells, while pericentral hepatocytes showed an increasing trend when TLR4 was inhibited. J Chain-high B cells ( $p=0.0424$ ), epithelial cells ( $p=0.0402$ ), and periportal hepatocytes ( $p<0.0001$ ) showed a statistically significant increase in cell proportions when STAT3 was inhibited. Both periportal hepatocytes and B cells (J Chain high) showed an increasing trend in cell proportions when TLR4 was inhibited, while epithelial cells showed no change in cell proportion. From the cell proportions in comparison with the 50 normal adjacent liver samples, the primary tumors saw significantly more hematopoietic stem cells ( $p=0.0041$ ), protumor B cells ( $p=0.0323$ ), myeloid dendritic cells ( $p=0.0461$ ), endothelial cells ( $p=0.0002$ ), fibroblasts ( $p=0.0308$ ), gamma delta T cells ( $p=0.0393$ ), and hepatocytes ( $p=0.0153$ ). There was also a statistically significant decrease in megakaryocytes ( $p<0.001$ ), naïve B cells ( $p=0.0198$ ), natural killer cells ( $p=0.0020$ ), and cholangiocytes ( $p=0.0008$ ).

**Conclusion:** The role of STAT3 and TLR4 in the prevention of HCC through the alteration of cell proportions is a promising avenue to investigate in NASH-affected livers.

**Keywords:** hepatocellular carcinoma, cell deconvolution, cell proportions

UPWARDS Summer Program

### **Combined Drug Efficacy in Preclinical Models of Colorectal Cancer**

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**Background:** Colorectal cancer is the third most commonly diagnosed cancer and the second leading cause of cancer death in men and women combined in the U.S. Albeit not widely represented in terms of cancer awareness, about 150,000 Americans are diagnosed with this disease and more than 50,000 die annually. Patients suffer from a change in bowel habits, diarrhea, constipation, discomfort in the abdomen among many other symptoms. Several treatment plans are available including but not limited to surgery, radiation therapy, chemotherapy, as well as targeted therapy. In this study, we sought to optimize targeted therapy more specifically in terms of a combination drug therapy. We hypothesize that combination drug treatment using blockades against cancer proliferation-inducing targets will lead to greater efficacy compared to single-drug therapy. The treatment focuses on inhibiting EGFR, a receptor prominent in cancer cells and promotes their growth, through panitumumab as well as blocking the amino acid transporter, ASCT2, utilized by cancer cells for rapid reproduction, using V-9302 and its variants, the CDP series.

**Methods:** As a preliminary characterization of the colorectal cancer cell lines utilized in the study, a BCA protein assay was conducted to quantitate protein concentration of each sample extracted. Using the results from the protein assays, a Western Blot was conducted to detect proteins related to EGFR and ASCT2 in the samples. The antibodies used were Phospho-EGF Receptor, EGF Receptor, ASCT2, Anti-GAPDH antibody, Anti-rabbit IgG HRP-linked Antibody. From there, the effects of panitumumab and V-9302 on viability of the cancer cells were observed through single-drug therapy at several dose ranges (0.01 - 1000 g/mL for Panitumumab & 0.001 - 100 M for V-9302) with a 48 hour incubation. Finally, to determine the efficacy of the combined treatment another cell viability assay was conducted using panitumumab and either V-9302, CDP3, or CDP7.

**Results:** In our Western Blot, of the five cell lines used (DiFi, Caco2, SNUC4, HT29, SW48) it was observed that the relative concentration of EGFR was particularly high in DiFi, and the concentration of ASCT2 was relatively high in HT29 and SW48. From our single-drug therapy using panitumumab, we were able to observe a sizeable decrease in viability in DiFi and showed minimal death in the other cell lines. In treatment using V-9302, DiFi was also much more sensitive compared to the other four cell lines in the study. The combined treatment showed a substantial decrease in viability when compared to the single-drug therapy and ranged between 92% to 98% cancer cell death.

**Conclusion:** These studies provide confirmation that a combined drug treatment lead to not only greater cancer cell death when compared to single-drug treatment, but also to a significant and reliable degree. In the future expanding through further trials as well as a bioinformatics analysis to characterize the cell lines used. Comprehending the relationship between Western Blot results and sensitivity to drug treatment can also be extremely beneficial. The results of this study prompt us to look further into combination therapy and its implication as an important form of anti-cancer treatment for patients who suffer from colorectal cancer.

**Keywords:** Colorectal Cancer, Combined Drug Therapy, Panitumumab, V-9302

UPWARDS Summer Program



Abstract Number: 154

### **Arthritis-irAE: Underlying mechanisms and Therapeutic targets**

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**Background:** Immune-checkpoint inhibitors (ICIs) have been widely used to treat various cancers by rejuvenating T cell responses to attack tumors. ICIs have been associated with immune-related adverse events (irAEs) that have the ability to affect nearly any organ in the body. This study focuses on arthritis-irAE which can significantly impact a patient's quality of life by causing joint discomfort and inflammation. In most cases, arthritis is treated with steroids, however, studies have shown that steroids can significantly abrogate antitumor immunity induced by ICI therapy. Recently, by analyzing synovial fluid from patients with arthritis-irAE, we demonstrated that treatment of patients with combined ICIs (CTLA-4 and PD-1 inhibitors) results in steroid-resistant inflammatory arthritis with enhanced T helper (Th)-17 cell signatures (Nat. Com. (2022)). However, to date, further knowledge of arthritis-irAE pathophysiology is limited due to the lack of a preclinical murine model.

**Methods:** C57BL/6 mice were immunized with Chicken Collagen Type II emulsified with Complete Freund's Adjuvant (CII/CFA) then injected with either PBS, PD-1 inhibitor, or combined ICIs. In some experiments, mice injected with combined ICIs were treated with antibodies targeting Th-17 related cytokines. At the endpoint of each experiment, we harvested spleen, synovium and bone from euthanized mice to perform flow cytometry (synovium and spleen) and histology (bone) analyses.

**Results:** Similar to humans, combined ICI therapy induced more severe arthritis in mice with enhanced Th17 cell signatures compared to PD-1 inhibitor monotherapy. Of note, treatment of arthritis-irAE mice with agents targeting Th17-related cytokines results in improvement in arthritis severity.

**Conclusion:** We created a pre-clinical murine model of arthritis-irAE that mimics the clinical setting. Like humans, we observed enhanced Th17 cell signatures in arthritis-irAE induced by combined ICIs in vivo. Implying that blocking of Th17 cell-related factors may have an essential role in improving the clinical outcomes of patients suffering from arthritis-irAE.

**Keywords:** Immune-checkpoint inhibitors, immune-related adverse events, inflammatory arthritis, Th17 cells

UPWARDS Summer Program

### **The Role of Ndufs4 and Slc2a1 on D4M-UV2 Melanoma Tumor Metabolism and Growth**

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**Background:** Brain metastases are a common and devastating complication of melanoma, which is the most aggressive form of skin cancer. We recently showed that oxidative phosphorylation (OXPHOS) is elevated in melanoma brain metastases (MBMs). Additional studies suggest that aberrant tumor metabolism may suppress immune responses in cancer. However, the role of increased OXPHOS in MBMs on the immune microenvironment is unclear. We hypothesize that elevated tumor OXPHOS negatively impacts the tumor-immune microenvironment in MBMs.

**Methods:** In order to test this hypothesis, we generated D4M-UV2 murine melanoma cells in which we inhibited OXPHOS by knocking out (KO) Ndufs4 using CRISPR technology. As a comparator, we also generated D4M-UV2 cells with KO of Slc2a1 to inhibit glycolysis. Metabolic effects of each gene KO were analyzed using the Seahorse MitoStress Test on cells growing in vitro. The oxygen consumption rate (OCR) was measured to assess OXPHOS and extracellular acidification rate (ECAR) to assess glycolysis. The Cell Titer Blue assay was performed to determine the effects of each gene KO on cell growth. Measurement of the cytokines secreted by each cell line is ongoing using the MSD Cytokine Array platform.

**Results:** KO of NDUFS4 resulted in 44.6% reduction of basal OCR compared to wildtype (WT) D4M-UV2 ( $p < 0.0001$ ), whereas SLC2A1 KO did not significantly affect OCR. KO of SLC2A1 resulted in a 69.8% reduction in ECAR compared to the WT D4M-UV2 ( $p < 0.0001$ ), while NDUFS4 KO had a 20.6% inhibitory effect ( $p = 0.0439$ ). Neither NDUFS4 KO nor SLC2A1 KO significantly inhibited in vitro cell growth after 24 hours compared to WT D4M-UV2 cells. Samples have been collected and submitted for cytokine array analysis; results are pending at this time.

**Conclusion:** Our results demonstrate that KO of NDUFS4 or SLC2A1 result in predominant inhibition of OXPHOS or glycolysis, respectively, in D4M-UV2 melanoma cells. KO of NDUFS4 or SLC2A1 did not impact the proliferation of D4M-UV2 cells in vitro. Together, these results demonstrate that KO of NDUFS4 and SLC2A1 effectively model inhibition of tumor OXPHOS and glycolysis, respectively, without eliminating cell viability. In the future, we plan to use these models to evaluate the effects of OXPHOS and glycolysis inhibition in vivo, including on the anti-tumor immune response in MBMs. These experiments will help determine if there is a rationale to develop combinatorial treatment strategies targeting tumor metabolism and immunotherapy for MBMs.

**Keywords:** Melanoma, Tumor Metabolism, Immunotherapy, Melanoma Brain Metastases, Immunosuppression

UPWARDS Summer Program

Abstract Number: 156

### **Fibronectin and FAK signaling regulate ARHI-induced autophagy**

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**Background:** Autophagy or “self-eating” is a process that degrades cellular proteins and impaired organelles, providing energy amongst times of nutritional detriment or in the appearance of stress. Our lab has reported that the tumor suppressor gene ARHI (Aplasia Ras Homolog Member 1), also known as DIRAS3, is decreased in over 60% of ovarian cancers and induces autophagy in ovarian cancer cells. The perseverance of DIRAS3-induced autophagy hinders ovarian cancer cell growth in ovarian cancer cells in vitro, however DIRAS3-induced autophagy sustains dormancy in vivo. Here, we explored whether Fibronectin (FN), an extracellular matrix, can rescue ovarian cancer cells from cell death that is induced by DIRAS3 in culture.

**Methods:** Cell viability of ovarian cancer cells with or without DIRAS3 expression were accessed using SRB assay. Autophagy markers (LC3 and LC3II) were evaluated with Western blot analysis in the ovarian cancer cells with or without DIRAS3 expression. Signaling pathways required for DIRAS3-induced autophagic cell death were explored with Reverse Phase Protein Array (RPPA) and Western blot analysis.

**Results:** We have showed that DIRAS3-induced autophagy was inhibited by FN in two human ovarian cancer cell lines (SKOv3 and OVCAR8). Re-expression of DIRAS3 decreased the phosphorylation of FAK and AKT that could be reversed by addition of FN. Treatment of ovarian cancer cells with defactinib, a FAK inhibitor, decrease their growth and colony formation

**Conclusion:** Overall, we conclude Fibronectin reduces DIRAS3/autophagy induced cell death through activation of FAK signaling pathway. FAK inhibitor partially blocks FN-mediated signaling and inhibits tumor cell growth in ovarian cancer cells

**Keywords:** Ovarian Cancer, Autophagy, Integrin, FAK, Fibronectin

UPWARDS Summer Program

Abstract Number: 157

### **Fibronectin and FAK signaling regulate ARHI-induced autophagy**

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Robert Bast Jr, Experimental Therapeutics, University of Texas MD Anderson Cancer Center

**Background:** Autophagy or “self-eating” is a process that degrades cellular proteins and impaired organelles, providing energy amongst times of nutritional detriment or in the appearance of stress. Our lab has reported that the tumor suppressor gene ARHI (Aplasia Ras Homolog Member 1), also known as DIRAS3, is decreased in over 60% of ovarian cancers and induces autophagy in ovarian cancer cells. The perseverance of DIRAS3-induced autophagy hinders ovarian cancer cell growth in ovarian cancer cells in vitro. Nonetheless, in vivo DIRAS3-induced autophagy sustains dormancy. Here, we explored how Fibronectin (FN), an extracellular matrix, to a certain extent, can rescue ovarian cancer cells from cell death that is induced by DIRAS3 in culture

**Methods:** Autophagy was induced by re-expression of DIRAS3 and conversion of LC3/LC3II was evaluated with Western blot analysis. Signaling pathways required for DIRAS3-induced autophagic cell death were explored with Reverse Phase Protein Array (RPPA) and Western blot analysis. Growth inhibition was evaluated by using SRB assay

**Results:** We showed that DIRAS3-induced autophagy was inhibited by FN in two human ovarian cancer cell lines (SKOv3 and OVCAR8). Re-expression of DIRAS3 decreased the phosphorylation of FAK and AKT. Further analysis revealed that addition of FN decreased the inhibitory effect of DIRAS3 on FAK and AKT. Treatment with defactinib, inhibited the growth of ovarian cancer cells and decreased their ability to form colonies.

**Conclusion:** Overall, we conclude from our data that certain factors in the tumor microenvironment, like fibronectin, can rescue ovarian cancer cells from DIRAS3-induced cell death and contribute to their survival.

**Keywords:** Autophagy, Fibronectin, integrin, FAK, ovarian cancer

UPWARDS Summer Program