The Nathan Schnaper Intern Program in Translational Cancer Research and ACS Diversity in Cancer Research Program 2023 Research Symposium

August 4, 2023
HSF-3 Seminar Room

UNIVERSITY of MARYLAND
MARLENE AND STEWART GREENEBAUM
COMPREHENSIVE CANCER CENTER
NATHAN SCHNAPER INTERN PROGRAM

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Cancer Moonshot preceptors
The Penn State PROMISE Program
NSIP alumni and MSTP student panelists and presenters
-AND-
The 2023 NSIP and ACS-DICR mentors!

NSIP
Nathan Schnaper Intern Program
in Translational Cancer Research

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For more information, go to http://www.umm.edu/NSIP

2023 NSIP/ACS-DICR Research Symposium

Friday, August 4, 2023
8:30 am to 5:00 pm
HSF-3 Seminar Room

Time	Program	Speaker	Mentor
8:00-8:30 am		**Breakfast**	
8:30-8:35 am		**Bret A. Hassel, PhD** *Director's Welcome*	
8:35-8:48 am	NSIP	**Kristine Jones** *The Effects of an ERK1/2 Substrate Docking Site Mutation on related MAP Kinase Pathways*	Paul Shapiro
8:48-9:01 am	NSIP	**Grant Thomas** *Targeting Glutamine Metabolism in Mesothelioma Cell Lines*	Ashkan Emadi
9:01-9:14 am	NSIP	**Charan Ravikumar** *Identifying therapeutic targets for improving T-cell function during immunotherapy*	Nevil Singh
9:14-9:27 am	NSIP	**Erika Carmody** *Identification of new minimal domain that modulates NFkB to improve Immunotherapy*	Nevil Singh
9:27-9:40 am	NSIP	**Sai Pranav Majeti Venkata** *Leveraging yeast genetics in Saccharomyces cerevisiae to identify SARS-CoV-2 host-pathogen interactions*	Matthew Frieman
9:40-9:53 am		**Coffee Break**	
9:53-10:06 am	NSIP	**Anirudh Saxena** *Investigating the impact of differential DNA methylation on the racial disparity observed in oral squamous cell carcinoma*	Daria Gaykalova
10:06-10:19 am	ACS-DICR	**Arryn Berroya** *Functional characterization of nuclear receptor NR1D1 on investigation of brain metastasis*	Min Yu
10:19-10:32 am	NSIP	**Rebecca Oluwasanmi** *Pediatric Vestibular and Balance Assessment: Identifying Relationships and Implications*	Victoria Marchese
10:32-10:45 am	NSIP	**Kaitlin Chung** *Effects of Exercise on Pain and Pain Catastrophizing in Patients with Cancer Undergoing Chemotherapy: A Pilot Randomized Controlled Trial*	Ian Kleckner

10:45-10:58 am	NSIP	**Shalet James** *Dietary Interventions During Chemotherapy Treatment: A Systematic Review of Feasibility, Safety, and Efficacy*	Amber Kleckner
10:58-11:11 am		**Coffee Break**	
11:11-11:24 am	ACS-DICR	**Eliya Behailu** *Testing the Therapeutic Potential of Novel TFEB activators in Parkinson's Disease using Patient-iPSC models*	Ola Awad
11:24-11:37 am	NSIP	**Margie Ester Barrientos** *Assessing the efficiency of a DHODH inhibitor (BAY-2402234) in glioma and DIPG cell lines*	Eli Bar
11:37-11:50 am	NSIP	**Carmela Sambells** *Bioengineering reporters for experimental access to prime edited neurons from patient-model brains*	Alexandros Poulopoulos
11:50-12:03 pm	ACS-DICR	**Jefferny Lin** *Amphiregulin Promotes Head and Neck Cancer Cell Proliferation Through an ANGPTL4-Dependent Pathway*	Michal Zalzman
12:03-12:16 pm	NSIP	**Arjun Rakheja** *ZSCAN4-Mediated Histone Acetylation Links Osteoblast-Related Genes and the Cancer Stem Cell Phenotype*	Michal Zazman
12:16-1:15 pm		**Lunch**	
1:15-1:28 pm	NSIP	**Owen Eby** *^{1}H, ^{13}C, and ^{15}N Nuclear Magnetic Resonance (NMR) Assignments of Calcium-Bound Mutant and Wild-Type Bovine S100B with TRTK-12*	David Weber Kristen Varney
1:28-1:41 pm	NSIP	**Riyan Campbell** *Testing the sensitivity of standard care chemo-therapies in three human KRAS mutated pancreatic cancer lines*	Rena Lapidus
1:41-1:54 pm	ACS-DICR	**Isabella Perez** *Mechanism of action of artemisinins in pediatric leukemias*	Curt Civin
1:54-2:07 pm	NSIP	**Eleftheria (Lea) Petratos** *High-Throughput Reproducible Scale Up Manufacturing of DART Nanoparticle Platform for Clinical Translation*	Anthony Kim Winkles & Woodworth
2:07-2:20 pm	NSIP	**Ryan Yim** *Endotoxin-Sequestering Nanoparticle Protein Coronas to Mitigate Inflammation in Cancer*	Ryan Pearson
2:20-2:33 pm		**Coffee Break**	

2:33-2:46 pm	NSIP	**Annalyse Belton** *Sensitizing Cancer to Cysteine Starvation Using Ferroptosis Inducers*	Charles Hong & Charles Williams
2:46-2:59 pm	NSIP	**Viviana Smart** *Does TTLL11-induced Tubulin Glutamylation Enhance Metastatic Phenotypes to Promote Breast Cancer Metastasis in Vitro and in Vivo?*	Stuart Martin & Michele Vitolo
2:59-3:12 pm	NSIP	**Safiullah Rifai** *Prostate Cancer: Effects of Combinatoric Treatments on Metabolomics*	Arif Hussain
3:12-3:25 pm	ACS-DICR	**Haider Hussain** *Testing a Role for HSC70 in Post-Transcriptional Gene Regulation*	Gerald Wilson
3:25-3:38 pm	NSIP	**Anne-Fleur Winter** *Elucidating the non-canonical downstream effects of Tristetraprolin expression in triple negative breast cancer*	Gerald Wilson
3:38-3:51 pm		**Coffee Break**	
3:51-4:04 pm	NSIP	**Collin Bast** *Targeting K-Ras using tethered MRTX-849 Galeterone in the context of Pancreatic Cancer*	A. Sasha Krupnick
4:04-4:17 pm	NSIP	**Jaylen Tyson** *Engineering the hinge region of SHAB to improve fragmentation resistance*	Helen Dooley
4:17-4:30 pm	ACS-DICR	**Jaden Queen** *Monitoring NKT Cell Trafficking in Response to Ovarian Cancer-Associated Lipids*	Tonya Webb
4:30-4:43 pm	ACS-DICR	**Alena McQuarter** *Investigating the effectiveness of natural VEGF inhibitors on iNKT cell activation*	Tonya Webb
4:43-4:56 pm	NSIP	**Brian Schattle** *CAR T-cell therapy for treatment of HIV related lymphoma*	Djorde Atanackovic
4:56 pm		**Bret Hassel** *Closing Remarks*	

Abstracts

(in speaking order)

Kristine Jones
Loyola University Maryland
Mentor: Dr. Paul Shapiro

The Effects of an ERK1/2 Substrate Docking Site Mutation on related MAP Kinase Pathways

The extracellular signal-regulated kinase (ERK) 1 and 2 proteins belong to the mitogen-activated protein kinase (MAPK) cascade involved in cell proliferation, differentiation, and survival. Mutations in this pathway lead to the deregulation of the ERK1/2 proteins which has contributed to a variety of cancers, including malignant melanoma. Previous studies in the lab identified a novel function-selective inhibitor that binds cysteine residues 271/254 of ERK1/2, located on the F-recruitment site—an important docking site of the protein. Subsequently, the CRISPR/Cas9 system was used to generate two mutated cell lines at C271/C254 (converting both cysteines to alanines) in A375 parent cells: a melanoma cell line containing a BRAF mutation. Prior research found the A375 mutated cell lines exhibited slower growth and colony formation compared to the parents. A possible explanation of the differences found between the mutations and parent cell lines could reside in the relationship between the ERK1/2 pathway and the Jun N-terminal kinase (JNK) and p38 pathways—two additional MAPK cascades that regulate apoptosis and the cell cycle. Immunoblots of p38, JNK, and ERK1/2 proteins, as well as their downstream substrates, were generated with the use of the stimulate phorbol myristate acetate (PMA) and the p38 inhibitor Doramapimod (BIRB796) and then quantified into graphs to compare the ratio of protein levels. Preliminary results reveal a lower level of the p-p38 protein in the ERK1 C271-/- cell line in comparison to both the parents and the other mutated cell lines; differently, a higher level of the JNK protein in the ERK2 C54-/- cell line in comparison to the parents was also observed. Although the results are inconclusive, further investigation of the downstream targets of these proteins will provide a clear understanding of the scope of crosstalk and redundancy between these signaling pathways.

Grant Thomas
Washington College
Mentor: Dr. Ashkan Emadi

Targeting Glutamine Metabolism in Mesothelioma Cell Lines

Malignant mesothelioma is a rare form of aggressive cancer that typically affects the lining of the lung, or pleura. Current treatment options include surgery, radiation, and pemetrexed/cisplatin chemotherapy; however, the 5-year survival rate is only 12%, highlighting the need for better treatment options. A promising therapeutic approach employs the depletion of exogenous glutamine which is vital for cancer cell proliferation and tumor growth. L-asparaginase is an enzyme which catalyzes the breakdown of circulating glutamine and asparagine from the blood. Due to the aggressive character and thus high metabolic rate of mesothelioma cells, we hypothesized that mesothelioma cell lines would be highly sensitive to glutamine depletion and that the FDA-approved asparaginase, Rylaze, would be an effective therapeutic option. We found that in two mesothelioma cell lines, Meso-1 and NCI-Meso-17, removal of glutamine from the media inhibited cell proliferation. Additionally, we established that Rylaze inhibited cell proliferation in a dose-dependent manner with mean IC_{50s} of 0.12 ± 0.049 μg/mL and 0.15 ± 0.074 μg/mL for Meso-1 and NCI-Meso-17, respectively. Notably, when media glutamine concentration was lowered from 2.0 mM to 0.25 mM, the IC_{50} of Rylaze in

both cell lines cells decreased, confirming that glutamine depletion contributes to the anti-proliferative effect of Rylaze. We also found that Rylaze induced Meso-1 cell death at a dose of 1 μg/mL ($p<0.01$) and significantly inhibited cell culture expansion at 0.1 μg/mL in both cell lines. We next assessed the impact of Rylaze on global protein synthesis using a puromycin incorporation assay and found that Rylaze significantly decreased mesothelioma cell protein synthesis. Finally, we investigated the potential of Rylaze to improve the anti-cancer activity of the standard of care chemotherapies, cisplatin and pemetrexed, and found that the addition of Rylaze did not potentiate the effect of either agent. Ongoing work is further investigating the anti-cancer mechanism of Rylaze and determining whether staggering Rylaze and chemotherapy treatments can result in drug synergy.

Charan Ravikumar
University of Southern California
Mentor: Dr. Nevil Singh

Identifying therapeutic targets for improving T-cell function during immunotherapy

Abstract: T-cells are essential immune cells involved in natural immunity to cancer as well as the mediators of effective tumor therapy. T cells are activated when their receptor (the TCR) senses a target and triggers robust intracellular signaling leading to gene expression and cellular changes. Therefore, it is well recognized that targeting signaling pathways in T cells can be an useful approach to improve tumor treatment. NFκB is one such critical transcription factor controlling survival and effector functions. Matson et al. (2020) previously identified a unique role for the T cell surface glycoprotein CD5 in post translationally regulating IκBα, which stabilizes NFκB. The mechanisms by which CD5 modulates NFκB and its molecular consequences in cells are not known. We hypothesize that CD5 regulates IκBα by either directly associating with it or indirectly regulating proteins that destabilize IκB, such as Cbl-b. To examine this, we optimized a CRISPR/Cas9 approach to knockout Cbl-b in a T cell line (BW5147 cells) and then observe if CD5 is still able to regulate IκBα. At the same time, using a more unbiased approach, we are immunoprecipitating CD5 domains to identify specific proteins that interact with it in T cells. Finally, we are performing RNA-seq to elucidate transcriptional changes in BW5147 cells overexpressing CD5 to understand how the CD5-IκBα-NFκB axis regulates overall cellular function. These convergent approaches will help identify the novel mechanisms by which CD5 modifies IκBα in order to better understand the regulation of T-Cell survival and effector functions. These insights can be further be applied to cancer immunotherapies such as adoptive T-cell therapy in order to improve their efficacy.

Erika Carmody
Fordham University
Mentor: Dr. Nevil Singh

Identification of new minimal domain that modulates NFkB to improve Immunotherapy

The adoptive transfer of T cells and CAR T cells can be used to treat certain cancers. While highly effective when first given, adoptive T cell therapies can often be the root of relapse in cancer patients due to T cell death, inactivation, or loss of function. The lack of long-term T cell maintenance in patients requires repeated adoptive T cell therapy, which is financially and physically costly for the patient. The goal of my project is to develop a way to improve the function and

survival of transferred T cells. Previously the lab found that T cells with high levels of a protein called CD5 can survive better, by acting on the IkB-NfkB signaling axis via unknown mechanisms. However, since CD5 also has negative consequences on T cell activation, we hypothesized that identifying the specific domains of this protein involved in enhancing IkB (and therefore stabilizing NFkB) would help us improve T cell function in Immunotherapy. First, we generated retroviral constructs which systematically deleted portions of the 495 amino acid long CD5 protein and overexpressed each of them in a T cell line (BW). Using intracellular staining and flow cytometry, we found that a minimum region of Serine-Rich 29 amino acids (New Min) in the cytoplasmic tail was sufficient to enhance IkBa. Using this CD5 New Min we did further studies that (a) mutated key residues to examine their contribution, (b) generated new delivery methods to treat T cells and (c) further fine mapped the absolute minimum domain. We found that a single Tyrosine (Tyr) in the 29aa stretch, but not any of the Serines was critical. A 12aa stretch including this Tyr was also sufficient for function. Finally, we generated a cell penetrating peptide (CPP) linked version of CD5 New Min, that can be rapidly delivered to T cells. One of these formulations rapidly increased IkB with an even higher efficiency than retroviral transduction protocols we used so far. We are now validating these findings in primary T cells as well as functional changes associated with this. Additionally, we plan to directly examine the survival of primary T cells during adoptive T cell therapy to confirm that the increase in IkB directly correlates with increased T cell survival of those expressing CD5 New Min. Taken together, we have identified a new approach to potentially improve adoptive immunotherapy and defined a previously unknown pathway that regulates NFkB, which is one of the most important transcription pathways in mammalian cells.

Pranav Majeti
University of Maryland College Park
Mentor: Dr. Matthew Frieman

Leveraging yeast genetics in *Saccharomyces cerevisiae* to identify SARS-CoV-2 host-pathogen interactions

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is the causative agent for COVID-19, has infected over 750 million people worldwide. The rapid emergence of new variants and concern over resistance to current therapeutics has increased the need for novel antiviral treatments. SARS-CoV-2 is a positive sense RNA virus composed of 16 nonstructural, 11 accessory, and 4 structural proteins. Nonstructural proteins are involved in the regulation of viral genomes while accessory proteins function as virulence factors required for pathogenesis. The yeast, *Saccharomyces cerevisiae*, has been widely used to understand eukaryotic cell biology and can be used to understand interactions between viral proteins and eukaryotic cells. We propose to use a growth assay to identify yeast proteins/pathways that are perturbed by SARS-CoV-2 proteins using a yeast suppressor screen. SARS-CoV-2 genes were cloned into a galactose inducible vector and transformed into yeast. Yeast expressing each SARS-CoV-2 protein were analyzed for growth rate to identify SARS-CoV-2 genes that when expressed caused yeast to grow slowly. We found that expression of the SARS-CoV-2 genes NSP4 and NSP5 caused a significant growth reduction in yeast. We then performed a classic suppressor screen to identify yeast proteins that functionally interact with either NSP4 or NSP5 using a yeast gene KO library. Galactose inducible plasmids expressing SARS-CoV-2 NSP4 and NSP5 were transformed into yeast KO library plated on galactose-agar plates. If a specific yeast protein is involved in causing the slow growth phenotype, a reversal of slow growth is seen in the event of its knockout. We have identified yeast strains containing genes that when knocked out no longer have a slow growth phenotype when NSP4 or NSP5 are expressed. Analysis of these genes are underway with subsequent confirmation of homologues in mammalian cells. From these studies, we hope to gain a better understanding of how SARS-CoV-2 proteins interact with eukaryotic cells. We hope to use this screen to identify therapeutic targets and develop broad-spectrum antiviral therapies for highly pathogenic coronaviruses.

Anirudh Saxena
University of Maryland College Park
Mentor: Dr. Daria Gaykalova

Investigating the impact of differential DNA methylation on the racial disparity observed in oral squamous cell carcinoma

Accounting for approximately 65% of head and neck cancers, oral squamous cell carcinoma (OSCC) is the 6th most common cancer world-wide and has a poor 5-year survival rate of ~50%. Recent epidemiological studies indicate that Black patients with OSCC have worse overall survival as compared to their White counterparts. This racial disparity is postulated to arise from a combination of socioeconomic factors and differences in tumor biology. As cancer progression is a multistep process involving interplay between tumor suppressor genes (TSGs) and oncogenes that can be regulated, in part, by epigenetic modulations, we posit that differential methylation of TSGs and oncogenes may serve as a molecular determinant of OSCC progression in Black patients. Towards this goal, we acquired clinicopathological data from The Cancer Genome Atlas (TCGA) database for head and neck cancer cases. We further curated our cohort to only include OSCC cases which were human papillomavirus (HPV) negative for the White cohort, and either HPV-negative or HPV-unknown for the Black cohort. We further balanced the cohorts for age, sex, stage, and smoking status. We then ensured that the survival difference observed in the greater population was maintained in our cohort. Multivariate analysis of our matched cohort showed that, of the clinicopathological characteristics, race is associated with poorer overall survival of Black OSCC patients. The current results are not statistically significant due to the small number of Black patients, but this limitation will be alleviated with the inclusion of additional Black patients from a UMB cohort we are also curating. We plan to conduct methylation analysis to identify TSGs that are hypermethylated in tumors from Black patients as compared to those from White patients and oncogenes that are hypomethylated, again comparing tumors from Black patients and White patients. Furthermore, we will correlate the differential methylation with gene expression levels, using TCGA RNA sequencing data and functional validations. Ultimately, we hope to identify epigenetically altered molecular determinants of racial disparity in OSCC.

Arryn Joeina Berroya
University of Maryland College Park
Mentors: Drs. Min Yu and Remi Klotz

Functional characterization of nuclear receptor NR1D1 on investigation of brain metastasis

Brain metastasis is a fatal recurrence of advanced cancer that affects 20% of cancer patients. Capturing comprehensive brain metastasis landscape is critical to the establishment of sufficient and effective anti-tumor strategies. Ongoing research efforts in our team aim to better understand the biology of tumor cells in human brain metastasis. Preliminary data, generated by single cell sequencing of human brain metastases highlighted a potential role of the nuclear receptor NR1D1. Indeed, NR1D1 activity seems to be correlated with proliferation, immune evasion, and metabolism of brain metastatic tumor cells. The activity of this nuclear receptor can be controlled by its ligand Hemin (HEME) and is critical in retaining control of the circadian rhythm. Our aim is to investigate the functional properties of NR1D1 itself through breast cancer cell lines. Breast cancer cell lines, SUM190, MDA-MB-231 and JIMT1 were used to study NR1D1 function and undergone multiple treatments with conditions that consisted of exposing HEME and a synthetic agonist in various incubation times while adjusting media with no FBS, essentially starving the cells from creating their own HEME. In publicly available data sets it was found that low NR1D1 expression correlated with increase of Triradylglcerols metabolism and high NR1D1 expression with increase of amino acids and peptide metabolism. In addition, our data suggests that NR1D1 activation leads to cell morphology changes and future experiments will determine whether NR1D1 influences tumor cell proliferation and immune invasion. This will assist in determining discrete phenotypes of brain metastatic tumor cells and new approaches for treatment.

Rebecca Oluwasanmi
University of Maryland, College Park
Mentors: Dr. Emily McCarthy PT, DPT & Dr.Victoria Marchese PT, PHD

Pediatric Vestibular and Balance Assessment: Identifying Relationships and Implications

Maintaining balance is crucial for safe participation in activities, and it involves the interplay of sensory input and motor output. While the relationship between lower extremity muscle strength and balance in children is established, the link between sensory input and balance is not well established. Vestibular dysfunction has been associated with poor balance, but studies on the relationship between vestibular function and balance outcomes in children are limited. This study aims to examine the relationship between vestibular function and balance outcomes in typically developing children. Ten typically developing children aged 6-17 years participated in the study. Vestibular function was assessed using the Pediatric Vestibular Symptom Questionnaire (PVSQ), dynamic visual acuity (DVA), video Head-Impulse Test (vHIT), and vestibular evoked myogenic potentials (VEMPs). Balance outcomes were measured using the Timed Up and Go (TUG), Functional Reach Test (FRT), and modified Sensory Integration in Balance (mCTSIB). Muscle strength, self-efficacy, and physical activity levels were also evaluated. Statistical analysis was performed using Pearson and Spearman correlations. Most children had normal vestibular function, although one participant exhibited vestibular abnormalities. Dynamic balance (TUG) was significantly correlated with baseline postural sway, while horizontal canal function (vHIT gain) was associated with condition 4 of the mCTSIB. Asymmetry in oVEMP signals was significantly correlated with the vestibular ratio in the mCTSIB. Ankle dorsiflexion and knee extension peak force were related to dynamic and static balance measures, respectively. No significant relationships were found between physical activity levels and measures of balance or strength. This study provides evidence of the relationship between vestibular function and balance outcomes in healthy children. The findings emphasize the importance of screening for vestibular impairment in children with balance deficits and conducting comprehensive balance assessments. Clinicians should consider vestibular deficits as potential contributors to balance dysfunction in children. Future research should explore similar relationships in children with neurological conditions, vestibular deficits, and balance difficulties to comprehensively understand the contributions of vestibular function to balance in various populations.

Kaitlin Chung
Cornell University
Mentor: Dr. Ian Kleckner

Effects of Exercise on Pain and Pain Catastrophizing in Patients with Cancer Undergoing Chemotherapy: A Pilot Randomized Controlled Trial

40% of Americans receive a cancer diagnosis, and 45% of patients with cancer experience pain and may develop negative beliefs about their pain, a psychological construct called pain catastrophizing. The management of cancer-related pain and pain catastrophizing requires a multidisciplinary approach. Exercise offers a promising and safe treatment with biological and psychosocial mechanisms. However, no studies examine how exercise affects pain and pain catastrophizing in patients with cancer. We hypothesize that exercise can improve pain and how patients conceptualize their pain by boosting self-efficacy and reducing hyperconnectivity between sensory (e.g., primary somatosensory cortex, thalamus) and affective (e.g., dorsal lateral prefrontal cortex, anterior insula) brain regions. This secondary analysis of a randomized controlled trial (NCT03021174) investigated the feasibility and preliminary efficacy of exercise on pain and pain catastrophizing during an exercise intervention in patients undergoing chemotherapy. Nineteen patients (50% breast, 30% gastrointestinal, 20% multiple myeloma or genitourinary) were randomized to a home-based aerobic and resistance exercise intervention or nutrition control for 12 weeks. Patients completed

Symptom Inventory, Pain Detect, and Pain Catastrophizing Scale questionnaires at pre-, mid-, and post-intervention. They underwent fMRI scans at pre- and post-intervention, results forthcoming. Exercise caused a large reduction in Symptom Inventory pain scores mid-(ES=1.4) and post-intervention (ES=1.0) and a small to moderate reduction in Pain Detect scores mid- (ES=0.3) and post-intervention (ES=0.5). Exercise also caused a moderate to large reduction in Pain Catastrophizing Scale scores at mid- (ES=1.5) and post-intervention (ES=0.5). Future studies should test for replication and validation in larger samples and continue examining the mechanisms of these effects. This work can ultimately help guide researchers and clinicians in reducing the burden of pain on patients with cancer.

Shalet James
Nova Southeastern University
Mentor: Dr. Amber Kleckner

Dietary Interventions During Chemotherapy Treatment: A Systematic Review of Feasibility, Safety, and Efficacy

Chemotherapy is a mainstay for cancer treatment. However, chemotherapy is associated with side effects that can significantly limit a person's physical and cognitive functioning, both reducing quality of life and leading to dose reductions of life-saving treatment. Dietary interventions may help manage and reduce the side effects of chemotherapy through biological and psychological mechanisms. While there is preliminary evidence supporting the importance of nutrition in cancer care, current guidelines are vague except for minimum calorie and protein intake to prevent malnutrition. The aim of this systematic review is to evaluate the feasibility, safety, and efficacy of dietary interventions on health outcomes in adults undergoing active chemotherapy treatment for cancer. We searched three databases—Embase, Scopus, and Cochrane—using a combination of MeshTerms related to nutritional interventions during chemotherapy. Among the 1295 articles identified, 36 unique studies met inclusion criteria and were included in the review. Individualized nutrition counseling (n = 15) and fasting (n = 9) were the most prevalent dietary interventions studied in the cancer population, predominately in breast cancer patients (n = 16). The most common clinical outcomes for these trials were weight change (n = 10) and nutritional status (n = 8). The results suggest early and/or individualized nutritional counseling offers the best patient outcomes while being safe and feasible (n = 17). Warranting further exploration, high-protein plant-based diets and Mediterranean diets were found to lower cancer-related fatigue, one of the most common side effects of chemotherapy (n = 2). Moreover, fasting-related interventions were moderately safe and slightly less feasible among cancer patients undergoing chemotherapy treatment due to the time restrictions and side effects of the diet such as headache and nausea (n = 4). Future diet intervention studies with larger sample sizes in diverse cancer populations are necessary to deepen the understanding of nutrition in cancer care, refine clinical practices, and support the development of targeted interventions that optimize patient outcomes and well-being.

Eliya Behailu
University of Maryland, College Park
Mentor: Dr. Ola Awad

Testing the Therapeutic Potential of Novel TFEB activators in Parkinson's Disease using Patient-iPSC models

Parkinson's Disease (PD) is the second most prevalent neurodegenerative disease in the US with over 90,000 diagnoses made every year. It is associated with the progressive loss of dopaminergic neurons causing both motor and non-motor symptoms. Despite the ongoing effort, there is no effective treatment for PD. Our lab studied the mechanisms leading to neuronal loss that can be targeted for therapy using induced-pluripotent stem cells (iPSCs) from PD patients with *GBA1* mutations, the most common genetic risk factor for PD. In PD patient iPSC-neurons, we found decreased nuclear expression and activity of the Transcription Factor EB (TFEB). TFEB regulates lysosomal synthesis and clearance pathways which are essential for the cell's disposal of autophagic substrates and protein aggregates. Therefore, TFEB is considered an important therapeutic target in PD. In this investigation, we tested the ability of a novel chemical compound (BC18) to reverse TFEB alteration in PD patient neurons. Using immunofluorescence and Western Blot analysis, we quantified TFEB expression in the iPSC dopaminergic neurons treated with BC18. Our data showed that treatment with BC18 increased TFEB expression and enhanced its nuclear localization in the PD iPSC neurons. Further analysis indicated increased TFEB activity in response to the treatment. Restoring TFEB activity in the PD dopaminergic neurons can facilitate the clearance of cellular waste and protein aggregates in neuronal cells, enhancing survival. Thus, these findings suggest that this class of novel compounds hold a promising therapeutic potential for future treatments of PD.

Margie Ester Barrientos
Stevenson University
Mentors: Dr. Eli Bar, Dr. Fahim Ahmad

Assessing the efficiency of a DHODH inhibitor (BAY-2402234) in glioma and DIPG cell lines

Diffuse intrinsic pontine glioma is a high-grade brain cancer that affects children of ages 5-9 years old. Due to the location and biological characteristics of the cancer, the mean survival rate of diagnosed patients is one year. Current treatments like radiation, chemotherapy, and surgery have been found to not improve the survival rate of patients, so researchers have been investigating the effects of DHODH inhibitors due to their involvement in cell proliferation and the de novo pyrimidine synthesis pathway. Studies have often found that DHODH inhibitors are more potent when administered with another drug. This suggests that a DHODH inhibitor (Bay-2402234) is not as potent by itself due to extracellular factors that may prevent the drug from inhibiting the enzyme and delaying the synthesis of pyrimidine nucleotides. To test if a thick extracellular matrix or a multi drug resistance pump (MDR) is preventing Bay from working, 08387, 913, and DIPG 24 cell lines were treated with Bay for various time points as spheres and lysates. While the relationship between drug resistance and DHODH inhibition cannot be determined due to time constraints, Bay-2402234 has shown to be efficient at blocking the de novo pyrimidine synthesis pathway – at least in the 08387 sphere culture. Future directions involve a repeat of this experiment with the addition of another DIPG cell line to observe the effects of Bay-2402234 in a wider array of cells.

Carmela Sambells
University of Maryland, College Park
Mentor: Alexandros Poulopoulos, PhD

Bioengineering reporters for experimental access to prime edited neurons from patient-model brains

Prime editing is a hybrid CRISPR and reverse transcriptase technique that is able to make single nucleotide changes in a genome. This precision provides the opportunity to analyze patient-specific model systems with disorders induced by point mutations, such as epilepsy. However, evaluating the efficiency of such edits is challenging due to non-specific downstream effects, which makes it difficult to determine the precise genetic identity of the edited cells. This project aims to develop an in vivo reporter of prime editing efficiency, enabling accurate distinction between edited and unedited cells both visually and through cell sorting. The approach involves designing, constructing, and cloning a variety of reporter options in plasmids whose functionality is verified by transfection in HEK293 and COS-7 cells. This project explores two mechanisms for assessing prime editing efficiency, including the conversion of BFP to GFP upon the mutation of a single nucleotide, and repairing a disrupted GPI-linked fluorophore to express extracellularly in successfully edited cells. The utilization of these reporters will render edited cells visually distinguishable and sortable with high specificity. This improved precision in prime editing techniques is anticipated to enrich the edited cell population, and ultimately, holds great potential for patient-specific gene therapy, particularly for genetically complex disorders such as epilepsy.

Arjun Rakheja
University of Maryland, College Park
Mentor: Dr. Michal Zalzman

ZSCAN4-Mediated Histone Acetylation Links Osteoblast-Related Genes and the Cancer Stem Cell Phenotype

Cancer stem cells (CSCs) represent a distinct subpopulation within tumors that possess exceptional capacities for self-renewal, tumor initiation, and metastasis. While conventional cancer treatments target the bulk of tumor cells, CSCs have special mechanisms that allow them to be more resistant to therapeutics, more likely to metastasize, and contribute to tumor recurrence. Previous research in the Zalzman lab has shown that the ZSCAN4 gene is activated in Embryonic Stem Cells (ESCs) and CSCs, and promotes essential biological processes such as telomere maintenance, cellular proliferation, and induction of cancer stemness. Data from our lab shows that ZSCAN4 is involved in many stemness-related pathways. Furthermore, ZSCAN4 was found to bind to the enhancers and promoters of genes in the pathway of bone differentiation. This is important as genes involved in osteoblast differentiation often exhibit a dual role in cancer development, tumor progression and aggression. In this study, we aimed to determine the effect of ZSCAN4 on gene expression and histone acetylation at genes involved in osteoblast differentiation. We conducted a comprehensive analysis integrating CHIP-seq data from human cancer cells and mouse embryonic stem cells (ESCs), as well as RNA-seq data from mouse ESCs. We analyzed chromatin immunoprecipitation, next generation sequencing (CHIP-seq) data generated from human cancer cells and aligned these data with mouse ESCs and RNA-seq data from mouse ESCs. By leveraging these datasets, we successfully identified a subset of genes targeted by ZSCAN4-mediated acetylation. Our research provides mechanistic insights into the role of ZSCAN4 in mediating histone acetylation at genes implicated in osteoblast differentiation, thereby elucidating potential regulatory links between normal bone development and CSC-mediated cancer processes. These findings offer novel perspectives on CSC biology and hold promise for the development of innovative therapeutic strategies targeting both CSCs and osteoblast-related pathways in cancer treatment.

Owen Eby
Mount St. Mary's University
Mentors: Drs. David Weber and Kristen Varney

^{1}H, ^{13}C, and ^{15}N Nuclear Magnetic Resonance (NMR) Assignments of Calcium-Bound Mutant and Wild-Type Bovine S100B with TRTK-12

S100s are a class of calcium-binding proteins that modulate diverse cellular functions and are involved in a variety of disease states. S100s consist of a dimeric structure and characteristic helix loop-helix, or EF-hand motif, resulting in calcium binding regions. An S100 protein of particular research interest is S100B, a 10.7 kDa, 91 residue protein that is implicated in malignant melanoma. This protein is recognized as a malignant melanoma biomarker, indicating more severe disease prognoses. S100B has been shown to be a p53 binding partner, reducing p53 activity, oligomerization, and subsequent tumor suppressive effects. This activity makes S100B a promising therapeutic target. However, S100 proteins exhibit a high degree of homology, so designing an S100B inhibitor that exhibits high specificity and binding affinity remains a challenge. S100B is known to exhibit significant allosteric effects following calcium and target binding and understanding this at atomic resolution will inform the design of specific and effective S100B inhibitors. Towards this end, backbone resonance assignments were solved for the wild-type bovine Ca^{2+}-S100B with and without the Cap-Z derived TRTK-12 peptide, which is known to bind to S100B. Backbone assignments were also solved for a Ca^{2+}-S100B D61A mutant bound to TRTK-12. This mutation replaces an aspartic acid involved in calcium binding, the loss of which aids in the study of the allosteric effects of calcium and protein target binding. 2D ^{1}H-^{15}N HSQCs showed large chemical shift changes for both wild-type and mutant when the TRTK-12 peptide is bound. Chemical shift assignments were completed using the standard suite of 3D NMR experiments, HN(CO)CA, HNCA, CBCA(CO)NH, HNCACB, HNCO, HN(CA)CO, and CC(CO), on an Avance III Bruker 600 MHz NMR spectrometer. The data was processed with the NMRPipe program, analyzed in CcpNMR, and assigned to 95-100% completion. The resonance assignment of these proteins lays the groundwork for further NMR studies of these systems, including ion binding, dynamics experiments, and drug-binding studies.

Riyan Campbell
Brown University
Mentors: Dr. Lapidus, Dr. Ciner, and Brandon Cooper

Testing the sensitivity of standard care chemo-therapies in three human KRAS mutated pancreatic cancer lines

Pancreatic cancer has a five-year survival rate of about 5-10%, making it one of the most aggressive carcinomas. Aberrant gene expression contributes significantly to clinical disease and overall patient survival. The KRAS gene is responsible for creating a protein that controls cell signaling and regulating cell growth and division. When mutated, KRAS stimulates cell proliferation leading to the formation of primary tumors. Previous studies confirmed that patients with a G12C mutation, which involves a change in the DNA base where the glycine position turns into a cysteine position, responded much better to a poly therapeutic regimen of using the drugs Abraxane and Gemcitabine than a regimen made up of three other Folfironox drugs, 5-Fluorouracil, Irinotecan, and Oxaliplatin.
Therefore, we hypothesize that specific variants of the KRAS mutation will have a more significant response to a multi-drug combination treatment scheme in vitro. Moreover, this treatment regimen is hypothesized to show differences in sensitivity among the different mutations. To test our hypothesis, we performed proliferation assays of three human pancreatic cell lines, MiaPaca2 (G12C mutation), Capan-1(G12-V mutation), and HPAF-II (G12D mutation). We subsequently analyzed the potency of each alone and in combination to see the impact on proliferation. Preliminarily, MiaPaca2, HPAF-II, and Capan-1 did show variably sensitivity to the single agents and their combinations. Overall, as previously hypothesized, the study proved that the three different cell lines/mutations responded differently and had different sensitivities to the tested drugs. Further studies will measure potentiation and different combinations of Folfirinox drugs in the different KRAS mutations to see if there is still no effect on the proliferation.

Isabella Perez
University of California, Berkeley
Mentor: Dr. Curt Civin

Mechanism of action of artemisinins in pediatric leukemias

Artemisinins (ARTs) are a group of synthetic drugs used to treat malaria with low/absent side effects. ARTs are active against human leukemias, in vivo and in vitro. Considering ARTs kill leukemia cells which are resistant to current antileukemic drugs, we developed a potent ART analog (ART838) for potential leukemia treatment. We are working to understand the antileukemic mechanism of action of ART838 to maximize its potential clinical utility against leukemias. ARTs generate reactive oxygen species (ROS) which are necessary for their antileukemic activity. We previously found that ART838 treatment of human pediatric acute myeloid leukemia (AML) cells causes upregulation of several genes involved in the cellular Unfolded Protein Response (UPR), including the activating transcription factor-6 (ATF6) gene. We hypothesize treatment of leukemia cells with ART838 induces cell death by activating the UPR. My project is to determine if ATF6 is necessary for ART838-mediated leukemia cell death. We used CRISPR/Cas9 technology to knockout (KO) ATF6 from a pediatric AML cell line (MV4;11). We then assessed the proliferation/survival, metabolic activity, and ART838-induced apoptosis of wild-type (WT) versus ATF6 KO MV4;11 cells and found no substantial differences due to ATF6 KO. Therefore, we conclude that ATF6 is not necessary/required for the antileukemic activity of ART838. Since it is possible that either or both of the 2 additional limbs of the UPR pathway (sensed by the genes PERK and IRE1) might compensate for the ATF6 KO, our next steps are to evaluate combined KO of ATF6, PERK, and/or IRE1. If combined KO does not affect ART838-mediated leukemia cell death, the lab will have disproven the hypothesized UPR involvement in the mechanism of action of ART838. The lab will pursue other potential mechanisms of ART838 action, with the goal of revealing novel molecules to target for leukemia treatment.

Eleftheria Petratos
Loyola University Maryland
Mentor: Dr. Nikhil Pandey, Dr. Jeffrey Winkles, Dr. Graeme Woodworth, Dr. Anthony Kim

High-Throughput Reproducible Scale Up Manufacturing of DART Nanoparticle Platform for Clinical Translation

Glioblastoma (GBM, grade IV glioma) is an aggressive, primary malignant brain tumor that has an extremely poor prognosis (~Median survival ~12-15 months post diagnosis). A hallmark feature of glioblastoma is tumor-cell invasion into tumor-adjacent healthy brain tissue, resulting in intractable tumor recurrence. Conventional cyto-toxic therapies are unable to treat the brain-invading tumor cells due to the blood-brain barrier (BBB) limiting therapeutic access. Accordingly, targeted nanoparticle-based therapies and concomitant novel BBB opening methodologies are currently an exciting avenue of clinical research towards treating brain-invading GBM cells and improving GBM patient outcomes. Our lab has developed decreased non-specific adhesivity, receptor-targeted polymeric nanoparticles (named DARTs) with specialized surface coatings including an antibody detecting the cell surface molecule fibroblast growth factor inducible-14 (Fn14), an emerging GBM drug delivery portal. Currently, one of the major factors hindering the clinical translation of promising nanotherapeutics such as DART NPs, is the difficulty in synthesizing batches of drug-loaded nanoparticles that show minimal variation in their physiochemical makeup and to do so in a reproducible manner for clinical applications. In this research work, we investigated the use of microfluidics to optimize polymeric as well as lipid-based DART nanoparticle platforms towards controllable and reproducible synthesis for scale-up and clinical translation. We evaluated the effect of microfluidic parameters on the physiochemical properties of DART nanoparticles (size, surface properties, and drug loading). Our current results indicate that polymer/drug ratio, presence of stabilizing surfactant, and the particular purification methodology adopted are critical factors in assembling stable, scalable DART

nanotherapeutics. Specifically, for a PLGA-PEG-based paclitaxel (PTX) loaded DART nano platform- we found that optimizing the initial PTX encapsulation amount is critical to assemble stable DART-PTX nanoparticles via microfluidic-nanoprecipitation-based methodologies. In conclusion, the work presented here provides new insights into methods for effective scale-up of therapeutic nanoparticles and supports the continued development of the DART nanoparticle platform for brain tumors.

Ryan Yim
Amherst College
Mentor: Ryan M. Pearson and Jacob Shaw

Endotoxin-Sequestering Nanoparticle Protein Coronas to Mitigate Inflammation in Cancer

Cancer patients are more susceptible to sepsis-related immune complications due to their immunocompromised condition. Amongst cancer patients, 3.7% of patients are diagnosed with sepsis and sepsis-related mortality accounts for 9% of cancer deaths annually. Lipopolysaccharide (LPS) is an endotoxin that originates from Gram-negative bacteria cell walls and plays an important role in the pathogenicity of sepsis by inappropriately signaling host innate immune responses, systemic organ damage, and death. Immune cells produce an abundance of antimicrobial biomolecules, such as cytokines, defensins, antimicrobial peptides, and proteins, to aid in clearing the infection and sequestering/inhibiting LPS; however, this often proves to be insufficient. Poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) have been frequently employed as drug delivery agents for chemotherapeutics. Moreover, our group demonstrated their inherently anti-inflammatory properties using *in vitro* and *in vivo* models of endotoxemia. Although these NPs mitigated the exaggerated innate immune response, they did not directly combat bacterial infection or circulating LPS. We used proteomics to analyze the composition of plasma proteins that interact with the NPs that form a protein corona. Lactoferrin (Lf), an antimicrobial and anti-LPS protein, was identified as highly abundant in the corona. We hypothesized that PLGA NPs could be coated with Lf to impart improved therapeutic activity by mitigating bacterial growth and LPS levels. We found Lf bound to the NP surface in a concentration-dependent manner and Lf content was between 1% to 30% of the total NP weight. Lf-coated NPs were non-immunogenic when assessed using bone marrow-derived macrophages. Future studies will focus on optimizing the anti-microbial activity of these NPs in addition to their ability to sequester LPS to benefit the efficacy of PLGA NPs for sepsis.

Annalyse Belton
Coppin State University
Mentor: Dr. Charles Hong

Sensitizing Cancer to Cysteine Starvation Using Ferroptosis Inducers

Ferroptosis is a cell death pathway predicated on iron dysregulation that is generating interest for its ability to treat multiple cancer types. Dysregulation of iron results in the production of lipid reactive oxygen species (lipid ROS), a hallmark of ferroptosis. GPX4 opposes the production of lipid ROS and is consumed in the reaction. SLC7A11 also known as X□ is a glutathione/cysteine antiporter that imports cysteine into the cell where it is converted into glutathione which is essential for the production of GPX4. Cysteine starvation results in the depletion of GPX4 and has been demonstrated

to trigger ferroptosis. However, difficulty with long term dietary cysteine starvation makes clinic translation difficult. Our lab has previously identified Ogremorphin (OGM), a highly specific small molecule inhibitor of GPR68, a G-protein–coupled receptor that senses extracellular proton concentration (pH). We have previously demonstrated that inhibition of GPR68 induces ferroptosis in glioblastoma cells. In this study, we used Erastin, a small molecule inhibitor of SLC7A11 and OGM to sensitize osteosarcoma (U2-OS) and colorectal carcinoma (RKO) to acute cysteine starvation. Previous work in our lab suggests U2-OS are sensitive to both Erastin and OGM. Conversely, RKO is resistant to ferroptosis, OGM, and Erastin. We used cell titer glo to show co-treatment with cysteine starvation and Erastin or OGM significantly decreases cell survival in comparison to either treatment alone. We validated our data genetically using shRNA mediated knockdown of SLC7A11 and GPR68. Using liperfluo, a lipid ROS reporter dye, we demonstrate that co-treatment of acute cysteine starvation and OGM or Erastin increased lipid ROS production more than either treatment alone within the cells. This data suggests co-treatment with OGM, Erastin, or other ferroptosis inducers with short term cysteine starvation may have greater clinical potential than long term cysteine starvation alone. These findings contribute further understanding not only to our knowledge, but the practices of chemotherapy.

Viviana Smart
University of Maryland, Baltimore County
Mentor: Drs. Stuart Martin and Michele Vitolo

Does TTLL11-induced Tubulin Glutamylation Enhance Metastatic Phenotypes to Promote Breast Cancer Metastasis in Vitro and in Vivo?

Triple negative breast cancer (TNBC) is known to metastasize very quickly. TNBC cells disseminate from primary tumor cells and can enter the bloodstream, where these cells are termed circulating tumor cells (CTCs). The CTCs may then attach to the endothelial lining of the blood vessels and invade or extravasate into nearby tissue. Once in a distant tissue, the TNBC cells may either remain dormant or begin to grow as metastatic cancer. Given the harsh environment of the bloodstream and heterogeneity of CTCs, this can lead to poor prognosis and higher risk of metastatic relapse for breast cancer patients. Therefore, it is critical to elucidate cellular mechanisms that promote the metastatic cascade to develop targeted drug therapies to inhibit and/or prevent metastasis.
To understand the metastatic potential of TNBC, the Martin lab has focused on the cytoskeletal components of the cells along with post translational modifications that affect the rate of metastasis. Metastatic breast cancer cells can have cytoskeletal membrane protrusions, also identified as microtenticles (McTN), caused by microtubule hyper stabilization. This is caused by the imbalance of microtubule growth overcoming the inward forces of the actin cortex. Microtubules are composed of polymerized alpha and beta tubulin heterodimers which are critical for cell structure and division. Post translational modifications on either tubulin heterodimer can enhance McTN growth and encourage cellular invasion and attachment during metastasis. Glutamylation on the C-terminus of alpha tubulin is a specific post translational modification that leads to microtubule stabilization and possible cellular invasion and reattachment. Tubulin tyrosine-like ligase (TTLL11) is a glutamylase that causes this modification and has implications in inducing breast cancer metastasis. The aim for the current project is to determine if expression of TTLL11-induced tubulin glutamylation promotes metastatic phenotypes in triple negative breast cancer cells in vitro and in vivo.

Safiullah Rifai
University of Maryland, College Park
Mentor: Dr. Arif Hussain

Prostate Cancer: Effects of Combinatoric Treatments on Metabolomics

Attacking DNA and DNA repair mechanisms are effective targets for prostate cancer. Cancer cells recruit non-homologous end joining, mediated by Poly ADP-Ribose polymerase (PARP), enabling the cancer cells to overcome DNA damage, while introducing mutations and contributing to genomic instability. Inhibiting Poly ADP-Ribose polymerase (PARP) in cancer cells increases double stranded breaks and cell death. Enhanced PARP activity converts Nicotinamide adenine dinucleotide (NAD) to Poly ADP-Ribose (PAR) chains, promoting DNA repair and affecting cellular metabolism. PARP inactivation increases NAD levels, impacting metabolic processes like glycolysis. Understanding metabolic changes due to PARP inhibition can lead to combinatorial strategies that compromise cancer cell metabolism, enhancing cell death. Preliminary data suggests synergism between PARP inhibitors and Nicotinamide phosphoribosyltransferase (NAMPT) inhibitors, which inhibit NAD production. These potentially altered NAD dynamics as a consequence of altered (enhanced or inhibited) PARP activity may affect cellular bioenergetics. Changes in aerobic respiration and glycolysis in LNCaP cells subjected to different treatments were observed using a Seahorse XFe24 analyzer. A NAD assay was also performed to assess potential changes in NAD in response to the different treatments. Western Blots were used to assess PAR levels (which reflects relative PARP enzyme activity) and the expression of γ-H2AX (a biomarker of DNA damage). Lastly, a Hoechst/PI assay was performed to verify relative cell viability. Preliminary results indicate that PARP inhibition shifts cellular bioenergetics towards glycolysis and combining NAMPT inhibitor reduces overall metabolism. Co-targeting PARP and NAD synthesis is expected to enhance antitumor activity.

Haider Hussain
University of Maryland College Park
Mentor: Dr. Gerald Wilson

Testing a Role for HSC70 in Post-Transcriptional Gene Regulation

Hsc70 is a member of the Hsp70 heat-shock protein family. It is a constitutively expressed RNA-binding protein and protein chaperone that has previously been shown to be upregulated in some cancer cell lines. Hsc70 shares similarities with its close relative, Hsp70, which is known to stabilize both proteins and mRNA through independent pathways. Hsp70 is also overexpressed in some cancer cells, which promotes cancer cell survival. There are major differences between the expression of the two proteins, as Hsp70 is normally stress-induced, while Hsc70 is constitutively expressed, suggesting that there may be fundamental differences in function. This project aims to determine the biochemical requirements for Hsc70 binding and the role of its RNA-binding activity in cancer cell survival. To determine Hsc70's specificity, we will use fluorescent anisotropy to compare its binding to AU-rich element-containing RNAs with other RNA substrates. Additionally, to determine which domains of Hsc70 are required for high-affinity binding to these RNA substrates, we will generate various truncation mutants of Hsc70, and test their RNA-binding activity through fluorescent anisotropy and electrophoretic mobility shift assays (EMSAs). Furthermore, we will investigate the impact of Hsc70 knockdown in cells using siRNA, where we will observe the protein's effect on the decay kinetics of mRNA targets. Here, we will utilize various techniques, including actinomycin D time course assays, qRT-PCR, and western blots. Understanding the structural and functional aspects of Hsc70, particularly its RNA-binding function, is vital in understanding its role in cancer cell survival, and could be important for potential therapeutics in the future.

Anne-Fleur Winter
University of Maryland College Park
Mentor: Dr. Gerald Wilson

Elucidating the non-canonical downstream effects of Tristetraprolin expression in triple negative breast cancer

Triple negative breast cancer (TNBC) is typically associated with high rates of metastasis and worse prognosis than other forms of breast cancer. TNBC has a very heterogenous molecular signature, hindering treatment development. Understanding the genes and pathways involved in its formation could provide targeted therapy options that reduce tumor growth and metastasis. One gene that is strongly downregulated in many cancers encodes the RNA-binding protein Tristetraprolin (TTP), suggesting functionality as a tumor suppressor. Our lab used three independent TNBC cell lines, MDA-MB-231, MDA-MB-436, and BT549, to stably express FLAG-tagged TTP. These TTP-expressing cell lines have decreased proliferation compared with their respective TNBC cell lines transfected with an empty vector. However, previous research has shown that TTP's known function, encouraging RNA degradation, is not the cause of tumor suppression in these cell lines. Determining the non-canonical mechanism of action of TTP's tumor suppression in TNBC would allow for novel treatment targets with future translational potential. To do this, RNA-sequencing was performed on these cell lines, and analysis of overlapping genes between RNA sequencing data sets generated a list of potential downstream effectors of the TTP tumor suppressor mechanism. qRT-PCR was then used to validate gene expression. qRT-PCR data elucidated three targets, HMGCS1, NDRG1, and LEPR as having decreases in RNA expression levels in TTP cell lines compared to those containing empty vectors. All three genes have roles in cell proliferation and high expression of these genes in patients is associated with decreased overall survival. The discovery of these intermediate effectors reveals new insights into TTP's RNA binding independent mechanism.

Collin Bast
University of Maryland, College Park
Mentor. Dr. Alexander Sasha Krupnick

Targeting K-Ras using tethered MRTX-849 Galeterone in the context of Pancreatic Cancer

The RAS GTPase protein is recognized as a key regulator of cell signaling and a driver of oncogenesis in approximately one third of all human cancers. Despite its significance as a therapeutic target, inhibiting KRAS has consistently proven to be ineffective. There is encouraging progress with an anti-RAS therapeutic MRTX849, specifically designed to target the G12C mutant KRAS. However, there have been instances of tumor resistance due to reactivation of the RAS pathway, posing a challenge that needs to be addressed for effective clinical application. We believe that the resistance to MRTX849 in KRAS-driven tumors arises from the depletion of mutant KRAS, which removes the negative feedback on EGFR signaling, consequently activating wild-type RAS and promoting tumor survival. In order to target and investigate this mechanism, our laboratory, in collaboration with Drs. Njar and Toth from UMB, have developed a tethered compound consisting of MRTX849 and Galeterone, a steroidal CYP17 and androgen receptor (EGFR) signaling antagonist targeting both EGFR and KRAS signaling. Our working hypothesis is that exposure to the tethered compound will decrease growth and viability, as well as downstream phosphorylation of KRAS mediators in G12C mutant cells. A pancreatic cancer cell line with the target G12C mutation in KRAS was exposed to varied concentrations of the tethered and individual compounds. Cell viability and growth were then assessed via a MTT assay, as well as flow viability measurements. Downstream signaling of KRAS and EGFR was examined following exposure to the individual drugs and tethered compound, by measuring phosphorylation levels of ERK 1/2 via flow cytometry. It was determined that the tethered compound inhibits the G12C KRAS mutant, and significantly decreases cancer cell viability with similar effectiveness to MRTX849. However, it did not suppress downstream phosphorylation of the ERK 1/2 protein over a period of 24 hours, indicating the possibility of secondary resistance. More research is needed to validate this secondary pathway, and determine whether it is mediated through EGFR.

Jaylen Tyson
Stevenson University
Mentor: Dr. Helen Dooley

Engineering the hinge region of SHAB to improve fragmentation resistance

The use of monoclonal antibodies (mAbs) has revolutionized cancer treatment by providing unique and effective targeted therapies. These antibodies exhibit diverse mechanisms of action, triggering potent anti-tumor responses while minimizing toxic effects. Shark VNAR human Fc fusion mAbs (shAbs) have potential for use *in vivo*, possessing the desirable effector functions and half-life of human antibodies, but with a much smaller binding site for practical use. However, the issue of instability arises due to fragmentation near the hinge region of these proteins during production, storage, and application. Previous studies have revealed that this fragmentation is the result of the presence of contaminating proteases and excess dissolved oxygen. To address this challenge, we hypothesize that reengineering the hinge region via the introduction of a point mutation and deletion of a protease binding site will result in increased stability. DNA sequencing has confirmed that the re-engineered short hinge was successfully introduced into the shAb HD1 vector. Future work will include comparing the stability of the short hinge with that of the original hinge region. By addressing the issue of instability through reengineering the hinge region, we anticipate a more reliable model for potential therapeutic applications in the treatment of cancer.

Jaden Queen
Cornell University
Mentor: Dr. Tonya Webb

Monitoring NKT Cell Trafficking in Response to Ovarian Cancer-Associated Lipids

It is estimated that >120,000 women worldwide die each year from ovarian cancer, thus it has the highest fatality-to-incidence of all gynecologic malignancies. Despite improvements in treatment, such as aggressive cytoreduction combined with chemotherapy, five-year survival rates of patients with advanced ovarian cancer remain less than 50%. The lack of effective treatment options for patients who relapse or have treatment-refractory disease requires the development of alternative interventions. It is known that in ovarian cancer, immune function is central to response to treatment and prognosis, and we have identified several factors produced by ovarian cancers that suppress host immunity. One potent immunosuppressive factor shed by ovarian cancers is the ganglioside GD3. We hypothesize that GD3 not only blocks NKT cell activation, but also suppresses the ability of NKT cells to migrate and intravasate into tumors. To test my hypothesis, we are developing an innovative platform wherein we label stromal cells, tumor cells, and NKT cells and observe their interaction in real-time using intravital spinning disk microscopy. By exploring the interaction between NKT cells and cancer cells, we can gain valuable insights into the immunological aspects of ovarian cancer and potentially identify novel mechanisms of immune evasion, which can ultimately result in the development of new therapeutic strategies.

Alena McQuarter
Arizona State University
Mentor: Dr. Tonya Webb

Investigating the effectiveness of natural VEGF inhibitors on iNKT cell activation

Ovarian cancer (OC) has the highest mortality rate of all gynecological malignancies. Given the fact that targeted and immune-based therapies have only had modest success, there is a critical need to develop innovative, effective therapeutic strategies. Natural killer T (NKT) cells are potent anti-tumor effector cells that are present within the OC microenvironment. However, studies from the Webb lab and others have demonstrated that OCs utilize mechanisms to inhibit NKT cell activation, proliferate and metastasize, such as the secretion of vascular endothelial growth factor (VEGF). Several natural herbal compounds have been shown to block the production of VEGF by cancer cells. Thus, we hypothesize that treatment with dietary supplements that inhibit VEGF will reduce OC growth and induce NKT cell mediated cytotoxicity. The goal of this study is to determine the effectiveness of natural VEGF inhibitors, such as those derived from compounds in green tea, licorice, and ginseng, in reducing the production of OC-associated immunosuppressive factors. To test these therapeutics, we will perform time course assays and dose-response curves using ID8, a murine OC cell line. We will measure cellular viability and VEGF levels following drug treatment. In future studies, we will investigate whether VEGF inhibition sensitizes OC to NKT cell-mediated killing. Collectively, these studies have the potential to lead to the development of novel therapeutic strategies for the treatment of OC.

Brian Schattle
Loyola University Maryland
Mentor: Dr. Djorde Atanackovic

CAR T-cell therapy for treatment of HIV related lymphoma

Patients that are diagnosed with HIV (human immunodeficiency virus) have a significantly increased risk for a future diagnosis of malignant B-cell lymphoma. Cancer cells often selectively change to avoid the natural immune response of a patient's T-cells. One way to treat B-cell lymphoma is through the use of CAR T-cell therapy. CAR T-cell therapy involves taking a patient's blood, and genetically altering the T-cells from the patient to fight the cancer cells. The T-cells are provided with a chimeric antigen receptor gene (CAR) that will bind to a protein on the cancer cells from the patient. This will allow the CAR T-cells to identify the infected cells and to release signaling molecules called cytokines to signal parts of the immune system to kill the cancer cells. In B-cell lymphoma, cancer cells present markers like CD19, and CAR T-cells can be provided with receptors to identify CD19 markers to ignite an immune response. As HIV negatively affects the immune system, patients that are diagnosed with HIV related B-cell lymphoma are not typically treated with CAR T-cell therapy and are excluded from clinical trials. We are looking at the CAR T-cell data through flow cytometry, blood counts, and clinical outcomes over several time points for two patients diagnosed with HIV related B-cell lymphoma and treated with CAR T-cell therapy. We will compare this data to controls. This research will report the experience of treating two HIV related B-cell lymphoma patients with CAR T-cell therapy to investigate the efficacy, safety, and effectiveness of treating patients with this diagnosis using CAR T-cell therapy.

Not presenting at the Symposium:

Simone Attles (ACS-DICR)
Hampton University
Mentor: Dr. Silvia Montaner

Role of Angiopoietin-like 4 in the resistance to cisplatin in HNSCC

Head and neck squamous cell carcinoma (HNSCC) is a group of cancers found in the oral cavity, pharynx, and larynx. Angiopoietin-like 4 (ANGPTL4) is a protein mostly known for therole of an adipokine involved in regulating lipid and glucose metabolism, but we do not know much about its relationship to cancer cells. The aim of this project is to study head and neck cancer cells that express Angiopoietin-like 4 (ANGPTL4) and their sensitivity to cisplatin. We are doing that by performing viability assays and comparing DNA damage when treated with cisplatin in ANGPL4 expressing and non expressing cells. This is an important aspect because prior research in my lab has suggested cells expressing ANGPTL4 combat DNA damage by either protecting the cells from damage or rapidly repairing the cell damage. This is why we are also completing comet assays to measure the amount of damage the cells undergo. Considering the amount of damage allows a clear determination of the impact ANGPTL4 has on cell damage in relation to cisplatin treatment and allows us to validate its significance. In both the viability and comet assays we have seen that cells that express ANGPTL4 undergo significantly less damage than cells that do not when treated with cisplatin. These results help to solidify the hypothesis that ANGPTL4 is either protecting the cells from damage or rapidly repairing the cell damage.

Eric Hudson (NSIP)
University of Maryland College Park
Mentor: Dr. Ciaran Skerry

Christina Cummins (MA-AUA Prep Intern)
Pennsylvania State University
Mentor: Dr. Minhaj Siddiqui