The Nathan Schnaper Intern Program in Translational Cancer Research and ACS Diversity in Cancer Research Program 2024 Research Symposium

July 26, 2024
Taylor Lecture Hall, Bressler Research Bldg

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

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NSIP alumni and MSTP student panelists and presenters
-AND-
The 2024 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

2024 NSIP/ACS-DICR Research Symposium

Friday, July 26, 2024
8:30 am to 5:00 pm
Taylor Lecture Hall

Time	Program	Speaker	Mentor
8:00-8:30 am		**Breakfast**	
8:30-8:35 am		**Bret A. Hassel, PhD** *Director's Welcome*	
8:35-8:47 am	NSIP	**Tamar Singman** *Biophysical screening methods for novel ERK1/2 inhibitors as possible chemotherapeutic agents*	Paul Shapiro
8:47-8:59 am	NSIP	**Angela Tan** *Regulation of human hematopoiesis by SIX1 and SIX2*	Curt Civin
8:59-9:11 am	NSIP	**Baani Singh** *Testing the Effect of Standard of Care Chemotherapies on KRAS-Mutated Pancreatic Cancer*	Rena Lapidus
9:11-9:23 am	ACS-DICR	**Sarah Benjumea** *The R-Ras/FLNA Complex is Robustly Expressed in Murine- and Patient-Derived Glioblastoma Cells*	Pavlos Anastasiadis
9:23-9:35 am	NSIP	**Katherine Groen** *Examining transcriptional suppression induced by interferon beta in neuroinflammation and cancer*	Darren Perkins
9:35-9:47 am	ACS-DICR	**Luka Ackerman** *Hypoxic memory: Regulation of hypoxic response by MBNL1 in glioblastoma multiforme*	Eli Bar
9:47-10:00 am		**Coffee Break**	
10:00-10:12 am	ACS-DICR	**Amaya Jones-Core** *Natural Supplements Can Reduce Ovarian Cancer-Associated Immune Suppression*	Tonya Webb
10:12-10:24 am	ACS-DICR	**Luis Quintero** *Developing CAR-NKT Cells for the Treatment of Brain Cancer*	Tonya Webb
10:24-10:36 am	NSIP	**Alli Jacobson** *Programming of tissue-specific tumor metastasis using chemokine receptors*	Nevil Singh

10:36-10:48 am	NSIP	**Riyan Campbell** *Characterizing the Differential Role OF M1 Macrophages in Primary and Metastatic PDAC*	Christina Ferrer
10:48-11:00 am	NSIP	**Sophie Werner** *Role of UBASH3B in Head and Neck Squamous Cell Carcinoma*	Daria Gaykalova
11:00-11:12 am		**Coffee Break**	
11:12-11:24 am	NSIP	**Ivan Fisher** *Efficacy of Exercise in Treating Chemotherapy-Induced Peripheral Neuropathy*	Ian Kleckner
11:24-11:36 am	NSIP	**Lauren Quick** *The Effects of a 10-Hour Time-Restricted Eating Pattern on Glucose Metabolism in Post-Treatment Survivorship*	Amber Kleckner
11:36-11:48 am	NSIP	**Lauren Savage** *Oxidative Stress: Role in the Development, Prognosis, and Functional Outcome of Cancer*	Tori Marchese
11:48-12:00 pm	NSIP	**Jeremy Ignatius** *The Activation of the Pluripotency Pathway in HNSCC Cancer Stem Cells*	Michal Zalzman
12:00-12:12 pm	ACS-DICR	**Kayla Mings** *Using cancer stem cell markers as targets for the development of therapeutic treatment for patients of African descent diagnosed with head or neck squamous cell carcinoma*	Michal Zalzman
12:12-1:10 pm		**Lunch**	
1:10-1:22 pm	NSIP	**Amogh Shetty** *Modeling the Temperature Profile of Indocyanine Green Based Photothermal Therapies*	Vikas Kundra
1:22-1:34 pm	NSIP	**Cole Vonderheid** *Artificial nanoparticle protein coronas as a novel anti-cancer therapeutic approach*	Ryan Pearson
1:34-1:46 pm	ACS-DICR	**Paula Delgado** *Development of CDK9 PROTAC-Encapsulated Liposomes for Acute Myeloid Leukemia*	Ryan Pearson
1:46-1:58 pm	NSIP	**Lily Handwerger** *Investigating the Mechanism of Thymine DNA Glycosylase in Active DNA Methylation*	Alex Drohat

1:58-2:10 pm	NSIP	**Benick Mbaya** *Assessing OVA and MHC-I Expression in LLC-OVA and B16-OVA Cell Lines*	Alexander S. Krupnick
2:10-2:25 pm		**Coffee Break**	
2:25-2:37 pm	NSIP	**Grace Acle** *Contribution of Specific Flanking Regions to the RNA-Binding Activity of the Protein AUF1*	Gerald Wilson
2:37-2:49 pm	NSIP	**Noa Deutsch** *Defining the anti-tumorigenic effects of tristetraprolin in triple-negative breast cancer*	Gerald Wilson
2:49-3:01 pm	ACS-DICR	**Sandy-Sheryl Angeh** *Impact of Novel Drug (VNPP433-3β) on Medulloblastoma Cells and Medullospheroids*	Aditi Banerjee
3:01-3:13 pm	NSIP	**Shylah Healy** *Functional single-cell tracking of brain cancer cells in culture*	Yajie Liang
3:13-3:25 pm	NSIP	**Cameron White** *The application of Oxaliplatin to enhance radiation efficacy on Glioblastoma cells via pSTAT3 inhibition*	Winkles/Woodworth/ Kim
3:25-3:37 pm	ACS-DICR	**Maia Pohlhaus** *ZNFX1's Role In Ovarian Cancer Progression*	Stajanovic/ Rassool
3:37-3:52 pm		**Coffee Break**	
3:52-4:04 pm	ACS-DICR	**Karleigh Landry** *Perforin-Independent Granzyme B Activity in Acute Graft-Versus-Host Disease*	Xuefang Cao
4:04-4:16 pm	NSIP	**Idrees Chaudry** *Disrupting the Function of Nucleocapsid within Virion Assembly Process in SARS-CoV and SARS-CoV2 by Inhibiting ROS from CNP Overexpression*	Matthew Frieman
4:16-4:28 pm	NSIP	**Linsy Song** *Understanding the role of circular DNA and host-defense peptides in pDC activation*	Lishan Su
4:28-4:40 pm	NSIP	**Clara Lopez-Ruiz** *Improving Retention of Shark-Derived VNARs through Human Serum Albumin Binding*	Helen Dooley
4:40-4:52 pm	NSIP	**Zabdiel Silva** *Metabolic Profiling of Bladder Cancer Organoids: Predicting Drug Response Using Patient-Derived Models*	Minhaj Siddiqui

4:52 pm **Bret Hassel**

Closing Remarks

Abstracts

(in speaking order)

Tamar Singman
Stevenson University
Mentor: Dr. Paul Shapiro

Biophysical screening methods for novel ERK1/2 inhibitors as possible chemotherapeutic agents

Constitutive activation of the extracellular signal regulated kinase 1/2 pathway (ERK1/2) pathway contributes to proliferative diseases such as cancer. Pharmaceutical inhibitors of kinase activity may be a potential chemotherapeutic avenue. In previous research, full kinase inhibition has been accomplished by drugs that block ATP binding. These results, however, do not lead to sustained clinical benefits. Complete inhibition of ERK1/2 prevents anti-proliferative negative feedback, which contributes to drug resistance over time. Additionally, the ATP binding site is conserved across many kinases, leading to potential off-target effects of ATP-competitive inhibitors. Alternatively, inhibition of pro-oncogenic signaling may be achieved by targeting ERK1/2 substrate binding sites. This approach may limit drug resistance and negative side effects. Our studies explore novel ERK1/2 modulators that target specific docking sites for substrates involved in cancer cell proliferation.

The biophysical properties of proteins may be used to examine their affinity for novel compounds. For example, evaluation of tryptophan fluorescence is an approach to evaluate molecular interactions. If a small molecule modulator interacts and changes the kinase's structure, tryptophan fluorescence quenching (FQ) can easily be measured by spectrophotometry. Another approach, referred to as differential scanning fluorimetry (DSF), involves changes in the melting temperature (ΔTm) of a protein upon interaction with a new compound. We hypothesize that FQ assays and DSF can be used to rapidly screen novel ERK1/2 modulators identified by computer-aided drug design (CADD). To test this hypothesis, we performed FQ and DSF assays on ERK2 with and without CADD identified compounds. Our studies indicate that FQ assays and DSF may provide preliminary screening data for our novel ERK1/2 modulators. These studies have implications in further research regarding rapid and cost-efficient screening methods for identifying novel kinase modulators as chemotherapeutic agents. Future directions will include examining the novel ERK1/2 modulators' anticancer properties in human melanoma cells.

Angela Tan
University of Maryland, College Park
Mentors: Dr. Curt Civin, MD; Dr. MinJung Kim, PhD

Regulation of human hematopoiesis by SIX1 and SIX2

Successful *ex vivo* expansion of a patient's own hematopoietic stem-progenitor cells (HSPCs) into mature blood cells could potentially mitigate immune incompatibility and infectious problems in blood cell transfusion and transplantation. Our lab is taking a molecular engineering approach toward developing *ex vivo* HSPC expansion strategies. Previously, we demonstrated that SIX1 overexpression (OE) in the human TF1 erythropoietic cell line model and in primary human $CD34^+$ HSPCs increased generation of erythroid cells (Creed *et al.*, *Development* 2020), and loss of SIX1 reduced human erythropoiesis. The human PAX-SIX-EYA-DACH transcriptional regulatory network (PSEDN) includes 6 protein isoforms (SIX1-SIX6) which can act as oncogenes in multiple types of solid cancers. Of the human SIX isoforms, SIX2 has the highest amino acid sequence homology with SIX1 (73%). Therefore, we hypothesized that SIX2 can also regulate human erythropoiesis. To test this hypothesis, we constructed lentivectors (LVs) to OE or CRISPR/Cas9 knockout (KO) SIX1 or SIX2. These four LVs contain a puromycin (Puro) resistance element and green fluorescence protein (GFP) to respectively select and identify transduced cells. We confirmed OE or KO by genomic DNA sequencing and Western blotting of the LV-transduced TF1 cells. Individual OE of either SIX1 or SIX2, even in the absence of erythropoietin (EPO), increased the numbers of TF1 cells expressing erythroid markers (increased CD71 and CD235a, decreased CD34, and increased hemoglobin). These results indicate that either SIX1 or SIX2 is sufficient to drive human erythropoiesis in the TF1 model. Planned studies will use these validated OE LVs to evaluate whether SIX1 and/or SIX2 OE can drive erythropoiesis of primary human $CD34^+$ HSPCs. We plan to go on to similarly evaluate the functional effects of SIX1 and SIX2 KO to determine whether SIX1 and SIX2 are not only sufficient but also necessary for human erythropoiesis, megakaryopoiesis, granulopoiesis, and/or monopoiesis.

Baani Singh
New York University
Mentor: Dr. Rena Lapidus

Testing the Effect of Standard of Care Chemotherapies on KRAS-Mutated Pancreatic Cancer

Pancreatic ductal adenocarcinoma (PDAC) is among the deadliest cancers; the 5-year survival rate is under 10%. With no screening test for PDAC and non-specific-presenting symptoms, patients are often diagnosed at advanced stages when surgery is not an option. The most common chemotherapeutic regimens for PDAC are 5-fluorouracil (5-FU), irinotecan and oxaliplatin (FOLFIRINOX), and gemcitabine and nab-paclitaxel (GN). MAPK pathway activation through KRAS mutations largely drives PDAC growth, invasion and metastatic spread. A real-world database analysis suggested that patients with KRAS G12V and G12D live longer with FOLFIRINOX, while those with G12C variants have improved survival with GN. Our project aimed to study this clinical observation in the lab using PDAC cell lines with G12D, G12V and G12C mutations respectively. We hypothesized that the G12C-mutated cell line would be relatively more sensitive to gemcitabine and/or nab-paclitaxel, while cell lines with G12D and G12V would be relatively more sensitive to 5-FU, irinotecan and/or oxaliplatin. First, we tested the individual drug's effect on each cell line, measuring each one's IC_{50} (concentration at which 50% of cells stop proliferating). We then tested the effects of the following combinations: 5-FU and SN-38 (the active form of irinotecan), 5-FU and oxaliplatin, and gemcitabine and nab-paclitaxel. The combinations did not show improved results compared to the single agents. It appeared, though, that one agent in each regimen impacted cell proliferation more dramatically. Future directions include testing these agents in more KRAS-mutated cell lines and testing the combination agents individually to better gauge each drug's effect.

Sarah Elena Benjumea
University of Maryland College Park
Mentor: Dr. Paul Anastasiadis

The R-Ras/FLNA Complex is Robustly Expressed in Murine- and Patient-Derived Glioblastoma Cells

R-Ras, a small GTPase involved in cellular signal transduction, and the cytoskeletal protein FLNA form a complex (R-Ras/FLNA) with the essential role of maintaining the integrity of endothelial homeostasis. The R-Ras/FLNA complex is most prominently present in the blood-brain barrier (BBB), protecting the brain from foreign entities. As in many different tumor types, the BBB is disrupted in glioblastoma (GBM). Due to the role that small GTPases play in GBM invasion and the integrity of the BBB, we investigated the expression of R-Ras and FLNA in different patient-derived GBM cells. We performed Western blot (WB) assays for the GBM cell lines GBM1, GBM6, and GBM39, probing for R-Ras, FLNA, and β-actin (loading control). Samples of 20 μg of murine-derived GL261 cells were used as a positive control for R-Ras and FLNA. We found that R-Ras and FLNA are expressed in GBM1, and R-Ras is abundantly present in GBM39, suggesting that R-Ras and FLNA could play an essential role in GBM cell migration and invasion. By determining the expression of R-Ras and FLNA in these cell lines, we aim to investigate the effects that the R-Ras/FLNA complex could have on the ability of GBM cells to migrate. We are particularly interested in this complex's potential impact on BBB permeability, which could significantly affect GBM treatment.

Katherine Groen
University of Maryland College Park
Mentor: Dr. Darren Perkins

Examining transcriptional suppression induced by interferon beta in neuroinflammation and cancer

Type I interferons (IFNs) are an integral part of the innate immune system and are known for triggering transcription of antiviral genes. However, in bacterial infections and some autoimmune conditions, IFNs are associated with immunosuppression, though the pathways are largely unknown. Understanding this paradox is clinically relevant as IFNs are used to treat multiple sclerosis and aggressive cancers such as melanomas. Previous RNA sequencing in bone marrow derived macrophages (BMDM) during a model inflammatory response identified several transcriptional pathways suppressed by IFNβ. The present study aims to leverage this dataset to determine if transcriptional suppression by IFNβ exhibited in BMDMs extends to cells exhibiting a neuroinflammatory phenotype and human cancer cells. Candidate genes were identified from RNA-seq based on decreasing expression with IFNβ pretreatment compared to an inflammatory stimulus alone. Afterward, qRT-PCR was performed to validate expression changes in BV-2, HeLa, and THP-1 cell lines. A secondary aim was to examine the effect of IFNβ on cell viability using a bioluminescent assay. Preliminary analysis found that some neuroinflammation-related genes were downregulated by IFNβ in both BMDMs and BV-2 microglia, while others did not show the same pattern. Cancer-related genes largely showed increased expression in HeLa and THP-1 cancer cells with IFNβ treatment, conflicting with expression changes observed in BMDMs. Lastly, we found that IFNβ incubation slowed HeLa cell growth while THP-1 cells showed no consistent change in growth. These results demonstrate that cell type and treatment conditions strongly influence IFNβ's impacts on transcription. Future directions should assess the biological impact of IFNβ treatment by modifying culture to model the tumor microenvironment and investigating protein changes. This work validates a subset of genes from a robust primary dataset and examines changes relevant to cancer and neuroinflammation, helping to elucidate the role of IFNβ in nonviral contexts.

Luka Ackerman
St. Mary's College of Maryland
Mentor: Dr. Eli Bar

Hypoxic memory: Regulation of hypoxic response by MBNL1 in glioblastoma multiforme

Muscleblind-like proteins (MBNL) belong to a family of tissue-specific regulators of RNA metabolism that control pre-messenger RNA-splicing (AS). Inactivation of MBNL causes an adult-to-fetal AS transition, resulting in the development of myotonic dystrophy. We have previously shown that aggressive brain cancer glioblastoma (GBM) maintains stem-like features (GSC) through hypoxia-induced responses and that hypoxia-induced responses in GBM also include MBNL-based AS that promote tumor progression. When cultured in hypoxia, GSCs rapidly export MBNL1 out of the nucleus, significantly inhibiting MBNL1 activity—notably, induced expression of MBNL1 results in a significant inhibition of HIF1-dependent signaling. To discover how MBNL1 regulates HIF1 signaling, we measured HIF1 signaling by 1) a reporter gene assay and 2) a quantitative polymerase chain reaction (PCR) of HIF1 target genes in wildtype GSCs and MBNL1 knockout GSCs cultured in normoxia and hypoxia. Our studies revealed a novel mechanism by which MBNL1 controls the magnitude and duration of HIF1 signaling.

Amaya Jones
Loyola University Maryland
Mentor: Dr. Tonya J Webb

Natural Supplements Can Reduce Ovarian Cancer-Associated Immune Suppression

Ovarian cancer (OC) has the highest mortality rates of all gynecological malignancies. Currently, it is challenging to identify shared molecular targets due to the heterogeneity in OC. Recent studies demonstrated that immune cell infiltration and OC survival are positively correlated with survival, despite the lack of effectiveness of immune-based therapy. Thus, our research aims are to identify the mechanisms underlying the immunosuppression caused by OC and to develop novel strategies to enhance anti-tumor responses to OC. The Webb lab previously demonstrated that OC produces vascular endothelial growth factor (VEGF) and ganglioside GD3, which can suppress immune cell activation. Therefore, we hypothesize that natural compounds that block the production of these factors will be more effective than other non-specific traditional therapies. To test our hypothesis, we treated 2D and 3D OC models with quercetin and polyphenol-60, natural compounds derived from fruit and green tea extract, respectively. Following treatment, VEGF-A levels were measured by ELISA. In addition, GD3 levels in human OC cell lines were assessed by dot blot and ELISA. Future studies will evaluate if treating tumor cells with these natural compounds can sensitize the OC cells to NKT-mediated cytotoxicity. Ultimately, these studies may lead to the development of new therapeutic approaches for the treatment of OC.

Luis Quintero
Davidson College
Mentor: Tonya Webb, PhD

Developing CAR-NKT Cells for the Treatment of Brain Cancer

Diffuse Intrinsic Pontine Glioma (DIPG) and Glioblastoma Multiforme (GBM) are aggressive brain cancers. The median survival time after diagnosis is low at only 8-14 months, thus the development of novel treatment strategies is urgently needed. Given that immune-based therapies have only had modest success, there is a critical need to identify unique molecular targets that block tumor growth and restore anti-tumor immune responses. For example, natural killer T (NKT) cells are potent anti-tumor effector cells. However, DIPG and GBM1 cells have been reported to express the ganglioside GD3, and studies from the Webb lab and others have shown that GD3 inhibits NKT and classic T cell activation, impeding immunosurveillance. We hypothesize that targeting GD3-expressing cells within the tumor microenvironment will restore anti-tumor immune responses and decrease tumor growth. In these studies, we characterized levels of GD3 expression in DIPG and GBM cell lines via flow cytometry, dot blot, and ELISA. We also assessed NKT cell activation utilizing lipid agonists, α-Galactosylceramide and 7DW8-5, using primary murine cells. Future studies will investigate whether DIPG and GBM cells directly inhibit NKT cell activation, or if blockade of CD1d-mediated NKT cell responses is the mechanism by which these brain cancers suppress NKT cell responses. In addition, we will investigate the efficacy of anti-GD3 CAR-iNKT cells as a novel therapeutic strategy for these recalcitrant tumors.

Alli Jacobson
Davidson College
Mentor: Nevil Singh, PhD

Programming of tissue-specific tumor metastasis using chemokine receptors

Cancer is a cellular disease where growth and proliferation are unregulated. When a mass of dysregulated cells separates from the initial site of disease and travels to a new location in the body, this is considered a tumor metastasis. Even when primary tumors can be treated and removed, metastatic masses may remain in some tissues. This is difficult to predict due to the erratic nature of disease progression and the variety of metastatic mechanisms. Currently, there is a distinct lack of appropriate models to replicate metastasis in a laboratory setting and predict which tissue a tumor may migrate to. Typically, cells navigate through the body using chemokine receptors (CR) that sense the “GPS coordinates” provided by chemokine signaling molecules in specific tissue sites. Therefore, we hypothesize that expression of specific CRs by tumor cells allows them to metastasize to certain tissue sites. Identifying which CRs predispose tumors to navigate to particular target tissues will allow us to (a) examine primary tumors early to predict potential metastatic venues, and (b) target CR signaling to prevent metastasis. In order to identify such homing mechanisms, we created a library of CRs and generated a panel of variant tumors, each which express a distinct chemokine receptor. CR-expressing tumor cell lines were also engineered to co-express both GFP and luciferase, which can be used to track the migratory properties of each variant both in vitro and in vivo. Using the approach, we generated lung cancer cell lines (LL2) with 12 CR-variants. These were FACS sorted on high GFP expression to establish 12 variant LL2 cell lines. Current experiments are examining how these variants migrate and if they have the propensity to metastasize to specific tissues.

Riyan Campbell
Brown University
Mentor: Dr. Christina Ferrer, PhD

Characterizing the Differential Role OF M1 Macrophages in Primary and Metastatic PDAC

The five-year survival rate for a patient with pancreatic cancer is 5 to 10%, classifying it as one of the most aggressive carcinomas. Specifically, pancreatic ductal adenocarcinoma (PDAC) is similarly known for not only its aggressive behavior but also its high likelihood of metastasis. One additional significant contributor would be the pivotal role of this dys-regulated gene expression. Consequently, from all of these factors, there is a dire need for novel therapeutics, and advanced diagnostics to significantly influence patient outcomes. Utilizing macrophages proves to be one promising and essential method to tackle this problem. It has been implied in recent studies that macrophages, specifically M1 macrophages, promote and inhibit cancer progression, solely depending on its tumor microenvironment. Metastatic gene expression pathways as well as invasion when pertaining to primary versus metastatic stages in (PDAC) are substantially affected by M1 macrophages. This then further explains the complex interplay and dynamics between the immune system and tumor progression. Enhancing anti – tumor immune responses highlight the potential of macrophages as a therapeutic target that will ensure patient outcome in survival. Herein, this study aims to determine the differential role of M1 Mac features in primary in metastatic (PDAC) . We will analyze whether there may possibly be a context- dependent role for M1 macrophages in primary versus metastatic (PDAC) by utilizing flow cytometry and QRT-PCR data analysis. Understanding the complex interactions between M1 Macrophages in the tumor microenvironments is essential for developing stage specific therapeutic strategies to combat PDAC metastasis.

Sophie Werner
Loyola University Maryland
Mentor: Dr. Daria Gaykalova

Role of UBASH3B in Head and Neck Squamous Cell Carcinoma

Head and neck squamous cell carcinoma (HNSCC) encompasses cancers of the epithelial lining of the oral cavity, pharynx, and larynx. HPV-negative (HPV-) HNSCC in African Americans correlate with a comparatively worse survival than European Americans with HPV- HNSCC. While the reasons behind this disparity are multifaceted, it is important to identify potential biomarkers to understand the underlying cause of this disparity. One possible candidate is ubiquitin-associated SH3 containing B (UBASH3B), a protein tyrosine phosphatase that stabilizes EGFR and correlates with proliferation and metastasis in various cancers. In this study, we used the clinical data from the Cancer Genome Atlas (TCGA) to investigate UBASH3B expression and its clinical outcome based on racial disparity. In addition, functional validation was performed to analyze UBASH3B expression in HPV- HNSCC cell lines at the mRNA and protein levels using qRT-PCR and western blotting, respectively. Both clinical and in vitro data revealed that UBASH3B was upregulated in HPV- HNSCC; African Americans with high UBASH3B expression had significantly worse survival compared to those with low UBASH3B expression. Further, qRT-PCR data revealed that loss of UBASH3B inhibited expression of key players involved in the UBASH3B-related pathways (EGFR-AKT pathway, EMT and cell cycle), thus, suggesting that the expression of these pathways is affected by the level of UBASH3B expression. In conclusion, our study reveals that UBASH3B expression affects the expression of target players involved in the UBASH3B-related pathways associated with cancer progression which can potentially affect clinical outcome of HPV- HNSCC in African Americans.

Ivan Fisher
Sattler College
Mentor: Dr. Ian Kleckner

Efficacy of Exercise in Treating Chemotherapy-Induced Peripheral Neuropathy

Over half of cancer patients receiving neurotoxic chemotherapy develop chemotherapy-induced peripheral neuropathy (CIPN), a dose-limiting toxicity involving numbness, tingling, and pain in the extremities, with up to forty percent of them experiencing chronic symptoms. These symptoms can severely interfere with daily activities such as walking, typing, or any tasks involving the hands or feet. Available drugs are largely ineffective against CIPN, highlighting the need for alternative options. Despite emerging evidence suggesting exercise as a promising treatment, it remains understudied how exercise affects CIPN symptoms and interference with daily tasks over time. We hypothesized that exercise performed during chemotherapy will mitigate CIPN symptoms over time and reduce interference with daily tasks. We enrolled 59 women with breast cancer receiving taxane chemotherapy in a 12-week study and randomized them to control (time- and attention-matched behavioral placebo, N = 25) and to Exercise for Cancer Patients (EXCAP, N = 24). Each day for approximately 12 weeks patients were asked to rate their neuropathic pain, numbness/tingling and interference on a scale of 1-10, with 10 being the highest you can imagine. Exercise caused a moderate to large reduction in neuropathic pain (ES = 1.03), numbness/tingling (ES = 0.92), and interference (ES = 0.98). Exercise also appeared to make similar neuropathic pain levels less interfering. Future work includes testing for replication with our complete study (N = 80), investigating further how exercise can make neuropathic pain less interfering, and examining the mechanisms by which exercise attenuates CIPN symptoms. This research could help optimize CIPN therapies, ultimately enabling cancer patients to maintain a higher quality of life while receiving essential treatments.

Lauren Quick
Community College of Baltimore County
Mentor: Dr. Amber Kleckner

The Effects of a 10-Hour Time-Restricted Eating Pattern on Glucose Metabolism in Post-Treatment Survivorship

Recent advances in cancer therapies have increased the length and quality of cancer survivorship. Glucose regulation is important in survivorship to reduce risk of cancer recurrence and chronic conditions, such as cardiovascular disease and metabolic syndrome, to which cancer survivors are prone. Time-restricted eating (TRE) is a dietary pattern that restricts the timeframe in which calories are consumed without restricting amount or composition of food. TRE benefits glucose metabolism in other health conditions, but has not yet been studied among cancer survivors. Thus, the purpose herein was to test whether TRE can improve glucose metabolism vs. control among cancer survivors. We recruited survivors 2 months-2 years post-treatment. All participants had dietary counseling then were randomized to TRE or control. The TRE group self-selected a 10-hour eating window for the 12-week study. Participants wore a continuous glucose monitor for 7 days during weeks 0, 6, and 12. Average, daily maximums and minimums, fasting glucose, and coefficient of variation were extracted from glucose traces. At baseline, correlations were assessed between glucose parameters and demographics and clinical characteristics. Then, linear regression models were developed to assess the effects of group on the glucose parameters at 6 and 12 weeks, controlling for baseline values. Data were available for 25 participants (age 57.5±11.5 years, 76% female, 88% blood cancers). Body mass index was positively correlated with average glucose (0.37±0.49, $p=0.46$). Interestingly, radiation was associated with higher average glucose and greater glycemic fluctuations vs. no radiation ($p<0.05$). At weeks 6 and 12, average glucose was higher for the control group than TRE, though the differences were not statistically significant ($p>0.05$). These results suggest that TRE is a promising nutritional program to regulate interstitial glucose among cancer survivors, especially those who have had radiation, though replication and a larger sample size is needed to validate our findings.

Lauren Savage
James Madison University
Mentor: Dr. Victoria Marchese

Oxidative Stress: Role in the Development, Prognosis, and Functional Outcome of Cancer

Redox status is the balance between oxidants and antioxidants, but an imbalance in favor of the oxidants results in oxidative stress. It has been established that an increase in oxidative stress promotes various pathological processes including cancer. In addition to being a risk factor for carcinogenesis, treatments such as chemotherapy and side effects such as cachexia can further disrupt cellular redox status, triggering muscle atrophy and other functional deficits which can manifest into loss of body weight, muscle, and fat, all of which are correlated with poor prognosis. Despite these deficits, there is little research demonstrating the effects of physical activity and rehabilitation interventions on oxidative stress and associated muscle atrophy in oncology patients. The aim of this study is to review the role of cellular redox balance and oxidative stress in both the advancement and prognosis of cancer. Assessments of oxidative stress in oncology patients were conducted in a literature search on PubMed. Five articles were selected using biomarkers that detected oxidant production, levels of antioxidants, and cellular redox balance across prostate cancer, sarcoma, breast cancer, leukemia, and non-small cell lung cancer. Levels of malondialdehyde (MDA), a measure of lipid peroxidation, and nitric oxide (NO), an oxidation product, were found to be significantly higher in cancer patients compared to healthy controls. MDA and NO levels were also elevated in patients with advanced prostate cancer compared to those with an early-stage diagnosis, and in lung cancer patients after receiving rounds of chemotherapy. Accordingly, antioxidant markers including total antioxidant capacity (TAC), glutathione (GSH), and superoxide dismutase (SOD) decreased in the same study groups. These findings support that altered redox pathways contribute not only to the development of cancer, but also to poor outcomes and increased treatment-related deficits. Future research should focus on the effects of rehabilitation interventions on oxidative stress and associated muscle atrophy in cancer patients, and on strategies that target maintaining cellular redox balance, which could be beneficial to improving overall survival and quality of life.

Jeremy Ignatius
Nova Southeastern University
Mentor: Dr. Michal Zalzman

The Activation of the Pluripotency Pathway in HNSCC Cancer Stem Cells

Over the last thirty years survival rates for Head and Neck squamous cell carcinoma (HNSCC) have remained constant at around 50%, despite great leaps made in the field of cancer treatment. Cancer stem cells (CSCs), a rare subpopulation within tumors, possess indefinite self-proliferation potential and are pivotal in tumor aggressiveness, invasion, and metastasis. These cells are highly resistant to conventional therapies such as radiation and chemotherapy, which makes them not only progress faster, also reoccur at higher rates. Targeting CSCs is therefore essential to planning modern cancer treatment plans as they play a key role in treatment resistance and cancer relapse. Previous studies from our laboratory have shown that the reexpression of the ZSCAN4 gene, known for its role in early embryonic development and telomere length maintenance, may contribute to increased cancer stem cell frequency in HNSCC. Our study aimed to confirm the expression of pluripotency markers such as OCT4 and NANOG and identify new stemness-related genes activated by ZSCAN4 in HNSCC. To evaluate the impact of ZSCAN4 on the pluripotency pathway, we used HNSCC cell lines (Tu-167) transduced with a tet-ZSCAN4 vector, and induced ZSCAN4 expression using Doxycycline. We then isolated RNA and performed cDNA synthesis, followed by Reverse Transcriptase Quantitative Polymerase Chain Reaction (RT-qPCR). The data from our RT-qPCR analysis validated the upregulation of the pluripotency pathway in HNSCC. Our research strengthens previous evidence linking ZSCAN4 to cancer stemness, and sheds light on previously unidentified new stemness genes activated by ZSCAN4, potentially providing new targets for future cancer treatment strategies.

Kayla Mings
Stevenson University
Mentor: Michal Zalzman, PhD

Using cancer stem cell markers as targets for the development of therapeutic treatment for patients of African descent diagnosed with head or neck squamous cell carcinoma

Head and neck squamous cell carcinoma (HNSCC) is a type of cancer that arises in the epithelial tissues of the head or neck excluding the brain. It is the seventh most common cancer globally, with over 800,000 new cases per year and a nearly 50% mortality rate. Despite its origin from a single cell type, HNSCC exhibits significant morphological heterogeneity. Current treatment selection for head and neck cancer (HNC) depends on tumor characteristics such as size, location, and observed metastasis into lymph nodes, aiming to remove the lesion while minimizing off-target cytotoxic effects. Advances in treatment have focused on both single modality and multimodal approaches, combining surgery with radiation to improve patient outcomes. However, despite these advances, more than 65% of patients diagnosed with squamous cell carcinoma eventually experience recurrence or metastatic disease. Racial disparities in HNSCC contribute to a worse prognosis and lower survival rate in the African descent population compared to white patients. These disparities are often attributed to factors such as later stages at diagnosis and inherent differences in tumor biology, however, the mechanism contributing to these differences are not well understood. Cancer stem cells (CSCs) are rare cells (1-3% of the population) within cancerous tumors that have unlimited proliferation capacity. These cells are highly resistant to conventional therapies such as radiation and chemotherapy and contribute to cancer relapse and metastasis. The aim of this study is to identify and compare the expression of stem cell biomarkers from HNSCC tumors from African descent and white patients. Identifying these markers may provide insights into the CSC niche aiding in the understanding of CSC and their contributions to HNSCC disparities. Our preliminary data reveal that HNSCC tumors from African ancestry patients exhibit a significant increase in multiple stem-cell-related markers. This suggests that CSCs may play a role in the observed disparities. Further research may lead to CSC targeted therapeutic strategies, in order to improve the diagnosis, prognosis, and treatment outcomes for patients of African descent.

Amogh Shetty
Rensselaer Polytechnic Institute
Mentors: Dr. Bonghwan Chon, Dr. Vikas Kundra

Modeling the Temperature Profile of Indocyanine Green Based Photothermal Therapies

In recent years, there has been greater interest in using indocyanine green (ICG) nanoparticles for photothermal therapies (PTT) against cancers. We sought to model the temperature profile of *in vitro* ICG based PTT in relation to the initial experimental parameters, such as dye concentration and laser power, and the fluorescence intensity over time. Using a thermal camera and a NIR camera, we simultaneously measured temperature and fluorescence in near infrared (NIR) I and II for various ICG concentrations in water and albumin solutions. We developed a program to process the raw data, generating data points every 270-750 milliseconds. Fluorescence and the rate of temperature change were modeled as monoexponential decay, yielding fitting of $R^2 > 0.95$. Model parameters were optimized using non-linear least squares and correlated with experimental conditions. As laser power increased, NIR II fluorescence and the rate of temperature change decayed faster ($p<0.001$; $p<0.001$). Conversely, as the initial ICG concentration was increased, NIR I, NIR II fluorescence, and the rate of temperature change decayed slower ($p=0.002$; $p<0.001$; $p<0.001$). The initial rate of temperature change was positively correlated with both laser power ($p<0.001$) and initial concentration ($p<0.001$). Using the linear correlations, we estimated model parameters for a testing set of experimental conditions. In 26 of 34 test samples, the predicted temperature profile had $R^2 > 0.8$. Lastly, the rate of temperature change could be modeled as a function of fluorescence with a single term polynomial ($n = 28/32$; $R^2 > 0.95$). Our mathematical models adequately predict the temperature profile in ICG based PTT depending on initial dye concentration and laser power, and as a function of the concurrent fluorescence. These models can aid in clinical implementation of PTT, such as in customizing initial parameters to reach the desired temperature conditions and tracking PTT mid-procedure with noninvasive fluorescence imaging.

Cole Vonderheid
Saint Mary's College of Maryland
Mentors: Dr. Ryan M. Pearson
Co-Mentor: Jacob Shaw

Artificial nanoparticle protein coronas as a novel anti-cancer therapeutic approach

Paula C. Delgado
The University of Texas at El Paso
Mentor(s: Dr. Steven Fletcher, and Dr. Ryan M. Pearson

Development of CDK9 PROTAC-Encapsulated Liposomes for Acute Myeloid Leukemia

Lily Handwerger
University of Maryland, Baltimore County
Mentor: Dr. Alexander C. Drohat

Investigating the Mechanism of Thymine DNA Glycosylase in Active DNA Methylation

The base excision repair (BER) enzyme, Thymine DNA glycosylase (TDG), is responsible for initiating the repair of G:T and G:U mismatches that result from deamination of 5-methylcytosine (5-mC) and cytosine, respectively. This is a critical function as cytosine to thymine mutations at CpG sites are prominent in cancer. Additionally, TDG contributes to gene expression by removing purposely modified bases for epigenetic regulation. 5-mC is an epigenetic marker associated with gene silencing. The process of removing 5-mC starts with ten-eleven translocation (TET) enzymes, which convert 5-mC to 5-hydroxymethylcytosine (5-hmC), leading to the formation of 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC) in further oxidation steps. Excision of 5-fC and 5-caC by TDG creates an abasic (AP) site, which is converted by BER enzymes back to cytosine, completing active DNA demethylation. 5-fC and 5-caC are excised through different mechanisms as they have different leaving group stability. Unlike 5-fC, excision of 5-caC involves acid catalysis and protonation of the caC base is thought to be facilitated by N191, given the massive reduction in caC activity caused by the N191A mutation. We are investigating the catalytic role of N191 using biochemical and other approaches. The N191A variant was expressed in bacteria, purified, and the mutational effect was determined using single turnover kinetics. The results indicate the mutation reduces activity for G:T and G:U substrates, and that it destabilizes TDG. Future directions include experiments with other mutants of N191, such as lysine (N191K) and aspartic acid (N191D), and additional methods including ^{19}F NMR studies of base flipping.

Benick Mbaya
Loyola University Maryland
Mentor: Dr. Alexander Sasha Krupnick

Assessing OVA and MHC-I Expression in LLC-OVA and B16-OVA Cell Lines

Grace Acle
Loyola University Maryland
Mentor: Dr. Gerald M. Wilson, PhD

Contribution of Specific Flanking Regions to the RNA-Binding Activity of the Protein AUF1

Many inflammatory and oncogenic factors are encoded by mRNAs that contain coordinately regulated *cis*-acting sequence elements, such as AU-rich elements (AREs). AREs and their regulatory proteins are often dysregulated in a variety of cancers. The ARE-binding protein AUF1 regulates the stability and translation of mRNA targets, and can be anti-tumorigenic or pro-tumorigenic in different cell contexts. AUF1 consists of four isoforms which result from alternative splicing of exons 2 and 7 from a single pre-mRNA. All isoforms have two tandem RNA recognition motifs (RRMs) that are the canonical protein:RNA interface. However, the inclusion of protein sequence from either alternatively-spliced exon alters binding, with the smallest isoform $p37^{AUF1}$ displaying the highest affinity for RNA. Mutants of $p37^{AUF1}$ lacking the N-terminal and/or C-terminal flanking domains display impaired binding, but an eight-residue motif from the N-terminal domain in addition to the RRMs and C-terminal domain was sufficient to restore most of the binding. My project interrogated the contribution of this short N-terminal motif and the similarly-positioned ordered motif found on $p40^{AUF1}$. $p40^{AUF1}$ includes the domain encoded by exon 2, located N-terminally of the RRMs, and contains an alpha-helix motif. Since the flanking regions on $p37^{AUF1}$ are predicted to be disordered, we hypothesized that this helical domain may account for $p40^{AUF1}$'s weaker binding. I tested a $p40^{AUF1}$ mutant with the helix removed and found a significant penalty in binding energy, suggesting that the isoforms employ different binding mechanisms. As AUF1 dysregulation is seen in a variety of cancers, this protein presents a potential therapeutic target if we could alter its binding and activity.

Noa Deutsch
University of Maryland, College Park
Mentor: Dr. Gerald M. Wilson

Defining the anti-tumorigenic effects of tristetraprolin in triple-negative breast cancer

Triple-negative breast cancer (TNBC) has an overall 5-year survival rate of 77%, which drops to 12% if the cancer metastasizes. Though targeted therapies have been developed and are highly effective for other breast cancer subtypes, TNBC is unique in that it does not express the receptor proteins that these therapies target, underscoring the urgency to identify novel protein targets. A new candidate is Tristetraprolin (TTP), an RNA-binding protein that has previously been described as a tumor suppressor in multiple cancers. To elucidate how TTP impacts tumorigenic phenotypes in TNBC, the Wilson lab stably transfected a FLAG-TTP-expressing construct into three metastatic TNBC cell lines and demonstrated that TTP induces several antitumorigenic effects *in vitro*. To determine whether these antitumorigenic effects of TTP observed *in vitro* translate to *in vivo* conditions, we created cell lines in a tumor-derived MDA-MB-231 cell line that constitutively and stably expresses FLAG-tagged TTP, as well as an RNA-binding mutant form of TTP called C147R. Cell lines were screened for FLAG-TTP expression, and clones exhibiting the highest levels of TTP expression will be used for further analysis. Additionally, we constructed inducible plasmids that encode FLAG-tagged TTP or C147R. We first cloned the tetracycline-responsive transcriptional activator (tTA) from the pTet-Off vector into the pcDNA3.1(+)/zeo vector. We then inserted the TREpminCMV promoter from the pTRE2hyg vector, and either FLAG-TTP or FLAG-C147R coding sequences. This single-plasmid system requires only one antibiotic for selection and will be used for further studies of TTP in TNBC, which is essential for advancing potential therapeutic strategies.

Sandy-Sheryl Angeh
Hood College
Mentor: Aditi Banerjee, PhD

Impact of Novel Drug (VNPP433-3β) on Medulloblastoma Cells and Medullospheroids

Medulloblastoma, originating in the cerebellum, is the most common malignant brain tumor in children accounting for 15-25% of pediatric brain cancers. While drug therapies are available, none are consistently effective at overcoming resistance and killing cancer stem cells (CSCs). As a result, there is an urgent need for a new therapeutic approach. This study investigates the efficacy of a newly developed compound, VNPP433-3β, and its molecular mechanism on medulloblastoma cell lines and medullospheroids derived from medulloblastoma cell lines. DAOY and D341 metastatic cell lines were used as in vitro models, and medullospheroids were used as an ex vivo model. The cells were cultured and treated with specific media containing growth factor, and their viability was assessed using the MTT assay to determine the IC50 value at 24 h, 48 h, and 72 h. Morphological assessment was conducted using an inverted microscope, and inhibitory properties were determined in a clonogenic assay. The MTT assays revealed a significant reduction in cell viability in a time and dose-dependent manner. Furthermore, treatment of cells with 1 μM of VNPP433-3β resulted in significantly fewer colonies and medullospheroids than untreated cells. The mechanism of cell death was examined using DAPI staining, which showed nuclear fragmentation and chromatin condensation, indicating cell death characteristic of apoptosis. Western blot analysis revealed that VNPP433-3β downregulated the oncoprotein FoxM1. Additionally, phospho β-catenin expression, associated with spheroid formation, was downregulated dose-dependent compared with untreated cells. These findings suggest that VNPP433-3β has the potential to be a therapeutic candidate for the treatment of medulloblastomas.

Shylah Healy
Johns Hopkins University
Mentor: Dr. Kevin Liang, PhD

Functional single-cell tracking of brain cancer cells in culture

Tumor heterogeneity, characterized by distinct changes between cells within the same tumor, is the main cause of drug resistance and relapse of cancers, including glioblastomas. We hypothesize that heterogeneity can be observed through functional single-cell tracking, as it can continuously observe a cell's behavior over a longer time frame. The development of the fluorescent ubiquitination-based cell cycle indicator (FUCCI) system has also expanded the approach to tumor heterogeneity, allowing for the visualization of the cell cycle phase in cancer cells. However, the blue fluorescent protein (BFP), conventionally used in imaging, is not ideal for in vitro single-cell tracking because it interferes with the strong autofluorescence from the culture medium. There are also no current sensors that can detect both calcium and the cell cycle, as calcium is an important second messenger for intracellular signaling pathways. We explored whether a new genetically encoded reporter construct can reliably report calcium and cell cycle progression through replacing the BFP in the FUCCI system with infrared fluorophore (miRFP670nano) to avoid the autofluorescence caused by the medium and incorporated a calcium indicator (GCaMP6s) to create the construct named functional FUCCI. We then packaged lentivectors and transduced HEK293 cells and performed long-term single-cell tracking through repetitive imaging of these cells at 15 min intervals for 24 hours. After image analysis, we successfully tracked the dynamic change in the fluorescence intensity of functional FUCCI regarding cell cycle progression and calcium dynamics in HEK293 cells. Initial analysis shows a wide distribution of migration speed and distance in the same population. Additionally, preliminary data from brain cancer cells (GBM1) expressing functional FUCCI cells show expression of red fluorescence, therefore implying the ability of GBM1 cells to undergo single-cell tracking and analysis for tumor heterogeneity. Future studies should be performed to evaluate tumor heterogeneity and if similar behaviors can be observed in differing cell lines or in vivo. Moreover, the quantification of such behaviors may eventually allow for discovery of new drugs and potential treatment to combat cancer relapse.

Maia Pohlhaus
Lehigh University
Mentors: Dr. Feyruz V. Rassool, PhD; Dr. Lora Stojanovic, MS, PhD

ZNFX1's Role In Ovarian Cancer Progression

Epithelial Ovarian Cancer (EOC) and more specifically High-grade serous ovarian carcinoma (HGSOC) is the most common EOC subtype that is still very difficult to treat due to an overall worse response to repeated courses of chemotherapy. EOC is thought to develop from the fallopian tube epithelium (FTE), where TP53 is the earliest event, followed by STIC lesions and BRCA mutations in some cases. The Rassool Lab has been studying the RNA/DNA sensor ZNFX1 (zinc-finger NFX1- type containing 1) in HSOC. Their current data collected from HGSOC samples as well as other cancers suggests that ZNFX1 expression increases with tumor stage and grade, making this gene important in disease progression. My aim this summer was to understand and describe exactly where in the earlier stages of disease progression we can see ZNFX1 expression. With this goal in mind, I confirmed ZNFX1 expression in two ways. Firstly, I collected samples of various cancers (lung, colon, ovarian) and asses ZNFX1 expression through western blots. My studies validated that there is varying and robust ZNFX1 expression between different cancer cell lines, emphasizing the importance of studying this gene. Secondly, I ran a qPCR of 6 different ovarian cancer mouse FTE cell lines with genetically defined mutations to see if any were associated with ZNFX1 expression. My qPCR results confirmed what was previously found in Dr. Rassool's lab that ZNFX1 expression is robust in ovarian cancer cells. My results in the genetically defined FTE cell lines were as follows: First, ZNFX2 has a much higher expression in BPPNM which has a BRCA mutation, compared with other genetically defined cell lines. Second, there was moderate expression in cell lines, KPCA.B with KRAS mutations and SPCA with Smarca4 mutations, respectively. This suggests interesting future directions focusing on performing experiments to determine whether ZNFX1 expression is mechanistically linked to BRCA mutations. Specifically, analyzing the role of ZNFX1 in homologous recombination repair that is defective BRCA mutant HGSOCs would be important.

Karleigh Landry
Xavier University of Louisiana (New Orleans, LA)
Mentor: Dr. Xuefang Cao

Perforin-Independent Granzyme B Activity in Acute Graft-Versus-Host Disease

Allogeneic hematopoietic cell transplant (allo-HCT) is a promising treatment option for hematologic malignances and nonmalignant blood disorders. However, this treatment often leads to graft-versus-host disease (GVHD). GVHD is amongst the main causes of death following hematopoietic stem cell transplantation. Granzyme B (GzmB) is vital for immune response, inducing apoptosis in aberrant cells and mediating autoimmune and inflammatory processes. We hypothesize that GzmB functions independently of Perforin in the modulation of acute GVHD. To study the difference of GVHD in the presence or absence of GzmB, BALB/c host mice were given hematopoietic cells from C57BL/6 donor mice to induce acute GVHD following allo-HCT treatment. We compared the effects of Wild-Type, Perforin Knockout CD8+, Perforin and Granzyme B Knockout (DKO) CD8+, Perforin Knockout CD4+, and Perforin and Granzyme B Knockout (DKO) CD4+ T cells on GVHD progression using a systemic grading system. Preliminary data indicates that Perforin KO CD8+ T cells induced severe GVHD compared with DKO CD8+ T cells, indicating that GzmB contributes to CD8+ T cell-mediated acute GVHD in Perforin-deficient setting. In contrast, Perforin KO CD4+ T cells induced less severe GVHD compared with DKO CD4+ T cells, suggesting that Perforin-independent GzmB activity in CD4+ T cells somehow diminish their ability to cause acute GVHD. Ongoing studies delve into inflammatory response and GzmB concentration between groups to explore potential impacts of Perforin-independent GzmB activity in both CD4+ and CD8+ cells. These findings could provide a better understanding of allo-HCT for hematological disorders.

Idrees Chaudry
Howard Community College
Mentors: Drs. Matthew Frieman, Stuart Weston

Disrupting the Function of Nucleocapsid within Virion Assembly Process in SARS-CoV and SARS-CoV2 by Inhibiting ROS from CNP Overexpression

Overexpression of a protein called 2', 3'-cyclic nucleotide 3'-phosphodiesterase (CNP) inhibits SARS-CoV-2 replication by localizing to and preventing depolarization of mitochondria. SARS-CoV-2 infection causes depolarization to release reactive oxygen species (ROS) from mitochondria, which is thought to aid in the viral nucleocapsid's (N) function in virion assembly. This project explores two hypothetical mechanisms by which inhibition of ROS release affects the N protein and disrupts virion assembly. The first hypothesis is that cytoplasmic ROS are required for N protein dimerization, which is required for its function. The second hypothesis is that cytoplasmic ROS are required for efficient binding of the N protein to the viral genome. We used transient transfection to express and study the N protein; however, this means there is no infection and no stimulus for ROS release. We therefore analyzed N dimerization status in the presence and absence of hydrogen peroxide (H_2O_2). If the N protein dimerizes in the presence of H_2O_2 but not in its absence, it would suggest that ROS release is required for dimerization, which could affect virion assembly. Using immunoprecipitation (IP) and qPCR, e can isolate the N protein and determine the amount of viral genome bound. We hypothesize that the presence of H_2O_2 would result in more viral RNA being captured, whereas in its absence, the N protein would have no or significantly less RNA attached. Ultimately, this suggests that inhibition of ROS release disrupts the binding of the viral genome to the N protein. A better understanding of the mechanism by which the later stages of viral replication are hindered can be used to develop more effective therapeutic treatments.

Linsy Song
Emory University
Mentors: Amara Ejikemeuwa and Lishan Su

Understanding the role of circular DNA and host-defense peptides in pDC activation

Our research focuses on which components in tumor and viral microenvironments can induce plasmacytoid dendritic cell (pDC) activation. pDC play a crucial role in the immune system, releasing robust amounts of type-I interferons (IFN-I) when activated through toll-like receptors (TLR-s). Typically, TLR-9 can sense single stranded microbial DNA. However, host-defense peptides like LL37 can interact with linear DNA to break tolerance of self-DNA and activate pDCs through TLR-9. Recently, extrachromosomal circular DNA (eccDNA), a product formed from DNA fragmentation in apoptosis, was found to act as a potent immunostimulant in macrophages and dendritic cells. Our research has also indicated that eccDNA has a more potent effect on pDC activation when compared to linear DNA, and LL37 strongly enhances this eccDNA, not linear DNA, mediated effect. Furthermore, previous research suggests that Interleukin-3 (IL-3) plays a complex role in the tumor microenvironment, influencing tumor progression and the immune response. We showed that when pDC were stimulated with IL-3 in conjunction with a TLR-9 ligand, CpG-A, at various time points, there was an increase in expression of IFN-I mRNA within 1 hour, but then a reduced response to CpG-A within 24 hours. In the future, we aim to understand how factors such as IL-3 may alter IFN-I expression in response to stimulants like eccDNAs and LL37, and the underlying pathways that allow for such outcomes. This knowledge could reveal new mechanisms of immune activation within tumor microenvironments and identify potential therapeutic targets.

Clara Lopez-Ruiz
University of Massachusetts Amherst
Mentor: Dr. Helen Dooley

Improving Retention of Shark-Derived VNARs through Human Serum Albumin Binding

Current cancer treatments include monoclonal antibodies (mAbs), which primarily function to block binding sites on molecules that promote cancer progression, flag cancer cells for destruction by the immune system, or deliver cytotoxic agents directly to cancer cells. However, mAbs are large and have inherent receptor binding abilities, which can limit their penetration into solid tumors. Shark-derived Variable New Antigen Receptors (VNARs) present a promising alternative to conventional mAbs. VNARs are 10-fold smaller than mAbs, thereby facilitating their tissue penetration, and possess high specificity and high affinity for antigens. However, a challenge in using shark VNARs as potential cancer therapeutics is their rapid clearance from the bloodstream due to their small size, leading to renal filtration within minutes. To address this, we hypothesize that linking therapeutic VNARs to anti-human serum albumin (HSA) VNARs will increase their retention time and enhance their therapeutic potential. To test this hypothesis, a nurse shark was immunized with a mix of HSA and SARS-CoV2 Omicron spike protein to trigger an immune response. Blood samples were collected from the immunized shark and the VNAR repertoire amplified from isolated PBLs to create a phage displayed VNAR library. This library was panned against HSA and mouse serum albumin (MSA), attempting to isolate any target-specific VNARs. After 3 pans individual clones were screened for binding by monoclonal ELISAs. Unfortunately, no target-specific clones were identified. Polyclonal ELISAs showed that enrichment of binders during panning was poor. Attempting to resolve this problem I screened clones from the unpanned library against both HSA, however this also did not yield HSA-binding VNARs. Given that the enhanced tissue penetration of VNARs could significantly benefit patients diagnosed with solid tumors, providing a novel and potentially more effective therapeutic option, work is ongoing to overcome this issue.

Zabdiel Silva
Johns Hopkins University
Mentor: Dr. Mohummad Minhaj Siddiqui

Metabolic Profiling of Bladder Cancer Organoids: Predicting Drug Response Using Patient-Derived Models

Bladder cancer is distinguished by metabolic characteristics that alter cellular responses to drug treatment. Identifying bladder cancer-specific metabolic profiles could consequently provide predictive insights into treatment effectiveness and specific metabolic characteristics could potentially be used to predict treatment response in patients. The focus of this study is the creation and use of organoids, which are three-dimensional models that accurately mimic the molecular and genetic features of human bladder cancers. To facilitate this, bladder cancer tissue was harvested from patients during surgical procedures, adhering to an IRB-approved protocol, and used to cultivate organoids. These organoids were cultured to provide a more precise simulation of the *in vivo* tumor environment. Utilizing the Agilent Seahorse XF analyzer, we measured key parameters such as oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) to monitor the dynamic processes of tumor evolution and assess tumor response to different therapies. This study utilized bladder tumor organoid lines harvested from individuals of African American (VA004) and European American (mBCA26 and mBCA36) descent to examine ethnic disparities in tumor behavior and treatment response, leveraging these detailed metabolic insights to potentially tailor therapeutic approaches. Upon administering Cisplatin, Gemcitabine, and UK5099 to organoid lines, we observed substantial differences in drug effectiveness and metabolic responses after 24 hours. UK5099 significantly reduced organoid viability by approximately 40% in the mBCA26 line. Gemcitabine led to a reduction in growth by 20-25% in the VA004 and mBCA36 lines. Metabolically, Cisplatin treatment elevated oxygen consumption by 15% in the VA004 line, reflecting increased mitochondrial activity. Gemcitabine decreased ATP production by 10-20%, indicating disruptions in cellular energy metabolism. Furthermore, reductions in glycolysis rates and glycolytic reserve capacity highlighted the metabolic adaptability among the treated lines. Our results suggest that personalized therapeutic methods could be developed based on individual metabolic characteristics of bladder tumors to improve treatment success.