

The Nathan Schnaper Intern Program in Translational Cancer Research and ACS Diversity in Cancer Research Program 2022 Research Symposium



August 5, 2022
SMC Campus Center – Elm Room B

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Dr. Armina Kazi and the Aromatase Inhibitor preceptors
Ms. Jennifer Aumiller and Dr. Hanover Matz
Cancer Moonshot preceptors
NSIP alumni and MSTP student panelists and presenters

-AND-

The 2022 NSIP mentors!



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

2022 NSIP Research Symposium

Friday, August 5, 2022

8:30 am to 5:00 pm

SMC Campus Center – Elm room B

Time	Program	Speaker	Mentor
8:00-8:30 am		Breakfast	
8:30-8:35 am		Bret A. Hassel, PhD <i>Director's Welcome</i>	
8:35-8:48 am	NSIP	Jameila Barrett <i>Investigating Metabolic Responses of Bladder Cancer Cells Treated with Therapeutic Agents</i>	Minhaj Siddiqui
8:48-9:01 am	NSIP	Isabella Cattaneo <i>Shark-derived nanobodies as potential cancer therapeutics</i>	Helen Dooley
9:01-9:14 am	NSIP	Kristen Zaranski <i>The Effect of Knocking Out NRF2 on Chemosensitivity in Pancreatic Cancer</i>	Rena Lapidus
9:14-9:27 am	NSIP	AyoOluwakiitan Oluwafemi <i>Analysis of in vitro and in vivo activity of the enantiomer of Edelfosine CP201</i>	Djordje Atanackovic
9:27-9:40 am	ACS-DICR	Carmela Sambells <i>Downregulation of TFEB Activity in Human iPSC Model of GBA1-Associated Parkinson's Disease</i>	Ola Awad
9:40-9:53 am	NSIP	Taariq Eddy <i>The Role of MHC II Expression on Immunologic Tolerance to Non-Hematopoietic Tumor</i>	A. Sasha Krupnick
9:53-10:06 am	ACS-DICR	Trinity Soto <i>Identification of transcription factors regulating NAG-1-P metabolism in rickettsiae by DNA affinity chromatography</i>	Joe Gillespie
10:06-10:21 am		Coffee Break	
10:21-10:34 am	NSIP	Phoebe Calkins <i>Specific Binding of Molecular Fragments to RBD1 of Clostridioides difficile Binary Toxin Identified by NMR and Site Identification by Ligand Competitive Saturation (SILCS)</i>	Adipietro/Weber/ Sevdalis
10:34-10:47 am	NSIP	Katherine Goundry <i>Tumorigenic Cells Evade Apoptosis in Free Floating Environments</i>	Stuart Martin

10:47-11:00 am	NSIP	Sona Kocinsky <i>Surveying Cancer Type Susceptibility to Ferroptotic Death Via GPR68 Inhibition</i>	Charles Hong
11:00-11:13 am	NSIP	Corinne Martin <i>Investigating a Mechanistic Link Between Neuronal Axon Projection and Brain Cancer Progression</i>	Alexandros Pouloupoulos
11:13-11:26 am	NSIP	Eva Gubitz-Hess <i>Investigating Optimal Expression Strategies of CRISPR Agents for Targeted Neural Somatic Genome Editing</i>	Alexandros Pouloupoulos
11:26-11:39 am	ACS-DICR	Eberechi Mbadugha <i>Amphiregulin Promotes Head and Neck Cancer Cell Proliferation Through an ANGPTL4-Dependent Pathway</i>	Silvia Montaner
11:39-11:52 am	NSIP	Collin Bast <i>Optimizing Immunocytochemistry Staining Protocol for the Co-Detection of Nuclear and Plasma Membrane Proteins</i>	Eli Bar

11:52-12:50 pm Lunch

12:50-1:03 pm	NSIP	Kyle Gunzy <i>Development of BDCA-2-targeting Antibody Using a Yeast Surface Display Human Antibody Library</i>	Lishan Su
1:03-1:16 pm	NSIP	Brianna Dewan <i>The Role of Nanoparticle Formulation and Purification Parameters on Macrophage Drug Delivery</i>	Ryan Pearson
1:16-1:29 pm	NSIP	Katie Donnelly <i>Targeting cytokine immunotherapy to the tumor using IL-12 subunits</i>	Nevil Singh
1:29-1:42 pm	NSIP	Erika Carmody <i>Improving CAR-T therapy by targeting signaling pathways regulating cell survival</i>	Nevil Singh
1:42-1:55 pm	NSIP	Jane Kimani <i>Natural killer T cell Responses to Lung Cancer</i>	Tonya Webb
1:55-2:08 pm	ACS-DICR	Aliqua Christensen <i>Monoglyceride Lipase Regulates Lymphocyte Activation</i>	Tonya Webb

2:08-2:23 pm Coffee Break

2:23-2:36 pm	NSIP	Pranav Majeti <i>Assessing the SARS-CoV-2 genome for slow-growth phenotype in Saccharomyces cerevisiae for the identification of potential therapeutic targets</i>	Matthew Frieman & Stewart Weston
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2:36-2:49 pm	NSIP	Darielle Runner-Draper <i>Effects of Altering FC Binding Region of HIV Antibody</i>	Alonso Heredia
2:49-3:02 pm	NSIP	James Hsia <i>Detection of HPV Integration Status in Head and Neck Cancer through PCR techniques and Characterization of Human-Viral Junctions in scRNAseq data</i>	Daria Gaykalova
3:02-3:15 pm	NSIP	Sophie Liu <i>ZSCAN4 knockout reduces in vitro cancer stemness in head and neck squamous cell carcinomas</i>	Michal Zalzman
3:15-3:28 pm	NSIP	Catie Corolla <i>Targeted inhibition of extracellular signal-regulated kinase 1/2 functions to inhibit cancer cells</i>	Paul Shapiro
3:28-3:41 pm	ACS-DICR	Christina Cummins <i>Targeted Inhibition of ERK 1/2 Functions in Large B cell Lymphoma</i>	Paul Shapiro
3:41 to 3:55 pm Coffee Break			
3:55-4:08 pm	NSIP	Rohaan Mahmud <i>Identifying Tumor-suppressive Mechanisms of the RNA-binding Protein TTP in Breast Cancer Cell Models</i>	Gerald Wilson
4:08-4:21 pm	NSIP	Patrick McGinnis <i>Roles of AUF1 Protein Domains in RNA Binding and Structural Remodeling</i>	Gerald Wilson
4:21-4:34 pm	ACS-DICR	Danielle Jones <i>The Impact of Bacteria and Metabolite Quantitation in Predicting Dental Caries</i>	Alison Scott
4:34-4:47 pm	ACS-DICR	Rebecca Oluwasanmi <i>Bordetella pertussis-induced IDO responses are age-dependent</i>	Karen Scanlon
4:47-5:00 pm	ACS-DICR	Tyonna Tyson <i>Developing a Bioinformatic Tool for Improved Prokaryotic Differential Expression Analyses</i>	Julie Dunning-Hotopp
5:00 pm		Bret Hassel <i>Closing Remarks</i>	

Abstracts

(in speaking order)

Jameila Barrett

Stevenson University

Mentor: Dr. Minhaj Siddiqui

Investigating Metabolic Responses of Bladder Cancer Cells Treated with Therapeutic Agents

Bladder cancer (BC) is a disease caused by the uncontrolled proliferation of malignant bladder tissue cells. Annually, 12,000 men and 4,700 women in the United States die from BC.¹ Previous literature indicated certain drugs may serve as possible therapies. Data has also shown African American (AA) patients have higher mortality rates compared to European American (EA) patients. This project aims to assess the metabolic responses of three BC cell lines exposed to different drug treatments, as well as to analyze metabolomic differences between EA and AA cell lines. In this study, cell lines T24 (EA), J82 (EA), and SCaBER (AA) were treated as follows: cisplatin (2 μ M), gemcitabine (2 μ M), metformin (10 mM), phenformin (20 μ M), cisplatin and metformin co-treatment (2 μ M and 10 mM). MTT cell viability assays were performed. For the three cell lines, the co-treatment decreased cell viability the greatest: 79% for J82, 54% for T24, and 43% for SCaBER. Comparison of the EA assays at 48 hours depicted that the drug treatments were less effective for T24. Metabolic activity was measured through extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) using Agilent Seahorse XF. ECAR measures glycolysis. Gemcitabine inhibited glycolysis by 80% for EA cell lines and 60% for the AA cell line, followed by cisplatin. OCR measures mitochondrial respiration. Phenformin inhibited respiration >80% for J82 and >99% for SCaBER. Metformin inhibited respiration >95% for T24. Our findings indicate that the drug treatments resulted in similar overall metabolic responses across EA and AA cell lines, but further experiments will need to be conducted to identify differences in altered metabolic activity. The co-treatment was shown to effectively decrease cell viability for all three cell lines. The higher cell viability for T24 may suggest the cell line is more aggressive. Gemcitabine and cisplatin exhibited the greatest inhibition on the glycolytic pathway, and phenformin exhibited the greatest inhibition on the mitochondrial respiratory pathway. Future studies will continue assessing drug treatments and comparing EA to AA cell lines for better metabolic characterization and ultimately mitigate poor patient outcomes.

Carmela Sambells
University of Maryland College Park
Mentor: Dr. Ola Awad

Downregulation of TFEB Activity in Human iPSC Model of *GBA1*-Associated Parkinson's Disease

Parkinson's disease (PD) is a common, progressive neurodegenerative disorder that causes motor dysfunction, cognitive impairment, and dementia. While clinical treatments exist for temporary relief, there is no available cure for PD. The scarcity of appropriate PD experimental models has hindered our understanding of the disease mechanisms and in turn, the development of new therapeutics. We took advantage of induced pluripotent stem cells (iPSCs) technology to generate neurons from PD patients harboring *GBA1* mutations, the most common genetic risk factor for PD. Using this model, we investigated the effects of the *GBA1* mutation on the transcription factor EB (TFEB), the master regulator of the autophagy-lysosomal pathway (ALP). The ALP is the cellular system responsible for the clearance of defective organelles and aggregate-prone proteins and its dysfunction leads to an accumulation of toxic proteins and neurodegeneration. We found that in PD neurons, TFEB expression and activity are reduced. Further analysis indicated that *GBA1*-mediated accumulation of cellular glycolipids upregulates the mammalian target of rapamycin complex 1 (mTORC1), the main upstream downregulator of TFEB. We hypothesize that lipid substrate-reduction compounds would restore TFEB activity in *GBA1* mutant PD neurons. To test this hypothesis, we treated iPSCs derived from PD neurons with Genz-123346 (GZ), a lipid substrate reducing compound and examined its effects on TFEB expression. Our results displayed that GZ treatment successfully reduced mTORC1 activity and restored TFEB expression in *GBA1* neurons as compared to untreated cells. Our study demonstrates that in *GBA1*-associated PD, lipid substrate accumulation deregulates the mTORC1-TFEB axis leading to ALP dysfunction. This suggests that targeting substrate accumulation may be a promising course of action for restoring TFEB activity in *GBA1* neurons for treating *GBA1*-associated PD.

Kristen Zaranski
University of Maryland College Park
Mentor: Drs. Rena Lapidus and Aaron Ciner

The Effect of Knocking Out NRF2 on Chemosensitivity in Pancreatic Cancer

Pancreatic cancer is an aggressive malignancy with a high mortality due to the difficulty of early detection and resistance to standard chemotherapy. Therefore, new approaches are necessary to identify chemo-sensitive subgroups and develop strategies to increase the efficacy of commonly used cytotoxic agents. NRF2 is highly expressed in pancreatic cancer and is thought to contribute to chemoresistance by neutralizing the production of oxygen radicals. Mechanistically, NRF2 reacts to oxidative stress by dissociating from KEAP1 and undergoing translocation to the nucleus where it is involved in the transcription of several downstream genes that regulate oxidative stress. This study examines the effect of knocking out NRF2 on chemosensitivity in three human pancreatic cancer cell lines: PANC-1, BXPC3, and MiaPaCa-2, and one murine pancreatic line: KPC/luc. We hypothesize that knocking out NRF2 will increase pancreatic cancer sensitivity to standard chemotherapy drugs. Western blot analysis demonstrated that NRF2 and its downstream genes are expressed in each cell line. In order to knock out NRF2, we designed CRISPR sgRNAs and PCR primers to nucleofect the cells and amplify the DNA for Sanger sequencing. We proceeded experimentation on only PANC-1 and KPC/luc. After successful nucleofection, we grew up bulk populations for the knockout cells and sequencing data validated a knockout population for all cell lines between 73-82%. We ran WST-1 assays to determine the IC₅₀ values for the parental and knockout cells to understand the effect of reducing expression of NRF2 on chemosensitivity. Additionally, we ran western blots to determine protein expression of NRF2 and downstream proteins in the parental and knockout cells. Preliminary results indicate that, opposite to our hypothesis, the sensitivity to chemotherapy of the knockout cells were either equal to parental or less sensitive. Western blot analysis showed no significant decrease in NRF2 in the knockout cells but did show a slight decrease in NQO-1 and increase in P-p65 expression in the knockout

cells. More research is needed to better understand alternate pathways that interact with NRF2, such as the NF- κ B pathway. It would be beneficial to continue studying the actions of NRF2 and associated molecular pathways to further understand these mechanisms and potentially develop new clinical applications.

AyoOluwakiitan Oluwafemi
University of Maryland, College Park
Mentor: Dr. Djordje Atanackovic

Analysis of *in vitro* and *in vivo* activity of the enantiomer of Edelfosine CP201

Synthetic Alkyl Lysopholipids (ALPs) are a unique group of molecules that present *in vitro* anti-tumor activity in several cancers including leukemia, breast cancer, pancreatic cancer, and more. Racemic mixtures of Edelfosine, an ALP, have been tested in phase 1 and 2 clinical trials where it has been shown to induce apoptosis in Multiple Myeloma (MM) cells while sparing healthy cells. Moreover, preliminary studies in the Atanackovic lab have shown that the enantiomer of Edelfosine, CP201, also induces apoptosis in MM cell lines. The goal of this study is to further evaluate the *in vitro*, and *in vivo* activity of enantiomer of Edelfosine, CP201, in combination with standard anti-MM agents. We hypothesize that combining CP201 with standard anti-MM agents will have a compounded effect on MM cells. To test this, we first aim to complete an *in vitro* assessment of antimyeloma activity of CP201. To this end, we have cultured MM cell lines RPM1 8226, MM1S, MM1R, and ANBL6 with increasing concentrations of CP201. Thereafter, we assessed the metabolic profile of the same MM cell lines treated with increasing concentrations of CP201. In addition, cell lysates of MM cell lines treated with increasing concentrations of CP201 were taken to assess apoptosis and stress. Next, we treated the same MM lines with increasing concentrations of standard anti-MM agents including Dexamethasone, Selinexor, and Bortezomib. Finally, we treated with CP201 and one of the standard MM agents. Based on findings from these studies, we will use the optimal combination of C201 with a second drug in *in vivo* studies. Our approach included staining cells with Annexin V and PI to assess apoptosis and cell death followed by flow cytometry. In addition, we used a assay and MTT assay to evaluate stress and apoptosis related protein concentrations and metabolic activity. Our Assays have shown a significant increase in apoptotic cells with increasing concentration of each anti-MM agent individually against each cell line. Furthermore, our assays suggested increasing concentrations of both CP201 and Selinexor, a standard anti-MM agent, against RPMI cell line has proven to greatly increase MM cell death and apoptosis. In the future, we hope to assess apoptosis and cell death in all our cell lines after being cultured with CP201 and another standard anti-MM agent. Finally, we hope to complete an *in vivo* assessment of the antimyeloma activity of CP201 in a xenograft mouse model using the most promising combination of treatment from the *in vitro* assessment.

Isabella Cattaneo
University of Notre Dame
Mentor: Dr. Helen Dooley

Shark-derived nanobodies as potential cancer therapeutics

Antibody-based therapies have revolutionized the clinical treatment of many cancers by activating the immune system's defense mechanism against them. For example, the monoclonal antibody trastuzumab is widely utilized in the treatment of HER2-positive breast and stomach cancers. While drugs such as trastuzumab have improved the prognosis for many patients, such therapies are limited by our ability to raise highly specific, high affinity antibodies against many human cancer targets. The Dooley lab is addressing this need through the immunization of nurse sharks with human cancer targets to raise novel shark-derived nanobodies (VNARs). These nanobodies are derived from the shark antibody isotype IgNAR, a heavy-chain only antibody, and have been shown to bind antigens with high specificity and affinity despite their very small size (1/10 that of a monoclonal antibody). These attributes make VNARs interesting prospects as future cancer therapeutics and diagnostics. While the small size of VNARs facilitate their biodistribution, these molecules have a short half-life *in vivo* and thus require some means of artificially increasing this to become useful therapeutically. The aim of this project was therefore to isolate a VNAR domain with high affinity for human serum albumin (HSA), a plasma protein which is very efficiently recycled *in vivo* and allows for a longer half-life. We can then fuse our anti-HSA domain to any cancer targeting VNARs to extend their *in vivo* half-life. To do this, I built a phage-displayed VNAR library using material from HSA-immunized nurse sharks. I then performed several rounds of panning to attempt isolation of HSA-specific VNAR clones. The results of this work will be presented and future plans outlined.

Tariq Eddy
Loyola University Maryland
Mentor: Dr. Alexander Krupnick

The Role of MHC II Expression on Immunologic Tolerance to Non-Hematopoietic Tumor Cells

One of the major barriers in tumor immunotherapy is the fact that malignancies are not significantly different from normal tissue. Malignant cells utilize naturally occurring mechanisms for downregulating the immune response to avoid immunologic recognition and destruction. Major histocompatibility class II antigens (MHCII) present exogenous material to CD4+ T cells and are key and central components of the immune responses. Usually this is only expressed by a set of cells defined as professional antigen presenting cells (APCs). Over the last decade the Krupnick laboratory has demonstrated that MHC II can be expressed on non-hematopoietic stromal cells not classically considered to be professional antigen-presenting cells. Furthermore, the function of MHC II on non-hematopoietic stromal cells is exclusively tolerogenic in nature. Here we hypothesize that MHC II expression on malignancies derived from stromal non-hematopoietic cells plays a tolerogenic role in downregulating the tumor immune response. A panel of murine tumors were evaluated using flow cytometry for expression of MHC II (fluorochrome conjugated anti-IA/IE, catalog #XXXXXX). Class II Transactivator (CIITA) is a master regulator was deleted from B16 murine melanoma using CrisprCas-9 gene editing according to standard methodology. None of the tumor cell lines expressed MHC Class II at rest however after stimulation with low dose IFN-gamma (50 ng/ml) both B16 melanoma and XXXX mesothelioma expressed detectable MHC II on their surface. However, no MHC II was detected on other cell lines such as Lewis Lung Carcinoma. B16 melanoma rendered CIITA null via CrisprCas-9 gene editing did not express surface MHC II. We have established methodology to test our hypothesis that expression of MHC II on non-hematopoietic cells plays a role in tumor-specific tolerance. Next steps include injection of wild-type and MHC II deficient B16 melanoma into immunosufficient and immunodeficient syngeneic mice in order obtain mechanistic data

Trinity Soto
Towson University
Mentor: Dr. Joseph Gillespie

Identification of transcription factors regulating NAG-1-P metabolism in rickettsiae by DNA affinity chromatography

Rickettsia is a genus of obligate intracellular bacteria in the Rickettsiaceae family that is transmitted by ticks, fleas, and lice. There is limited knowledge of the regulation of their metabolic pathways living in host cells. *Rickettsia* genomes lack glycolysis enzymes making them host-dependent bacteria; *Rickettsia* can make Lipopolysaccharides (LPS) and Peptidoglycan (PGN) only by stealing metabolites— N-acetylglucosamine-1-P (NAG-1-P) from a host. Gene regulation is not well studied, and the obligate intracellular nature of *Rickettsia* creates difficulties in genetic modification.

In our lab, a *Rickettsia* metabolic network was created as a general outline of how *Rickettsia* steals sugar from the host by utilizing the unique protein GlmU. GlmU is an enzyme that produces UDP-N-acetyl- α -d-glucosamine (UDP-NAG), an amino sugar that feeds into the LPS/PGN biosynthesis pathways from NAG-1-P. Curiously, there is an overlap of a putative transcription factor YebC with GlmU, which suggests that YebC could be a potential transcription factor in the *Rickettsia* metabolic pathway for LPS/PGN synthesis. The enzyme MurA was also analyzed since it produces peptidoglycan precursors from GlmU-generated UDP-NAG. We hypothesize that YebC will bind to its own promoter and the promoter of MurA.

To test the role of the YebC putative transcription factor in *Rickettsia*, cell cultures of *Rickettsia rhipicephali* were grown in Vero cells, *Rickettsia* isolated, and native protein samples were created for *in vitro* promoter binding assay. Native samples will be run through an SDS-PAGE gel and resultant proteins will be submitted for peptide mass spectrometry.

The expected results are a YebC band at 28 kDa on the SDS-PAGE for binding to the promoter of YebC indicating autoregulation and the promoter of murA indicating a potential regulon structure. These results will provide information for a better understanding of the regulation of the *Rickettsia* metabolism and the creation of vaccines for the treatment of infectious diseases.

Phoebe Calkins
Towson University
Mentor: Spiridon Sevdalis

Specific Binding of Molecular Fragments to RBD1 of *Clostridioides difficile* Binary Toxin Identified by NMR and Site Identification by Ligand Competitive Saturation (SILCS)

Clostridioides difficile (*C. difficile*) is a bacteria responsible for many nosocomial infections, particularly in cancer patients and those who are immunocompromised. Treatment options for the *C. difficile* infection are limited, and rates of infection have increased over the past twenty years. Particularly, virulent strains contain *C. difficile* binary toxin (CDT). This toxin has two subunits, CDTa which is the enzymatic component and CDTb, which acts as a translocase. This translocase allows CDTa to enter the cytoplasm and ADP-ribosylate G-actin, subsequently destabilizing the cellular cytoskeleton. The purpose of this project was to identify molecular fragments which bind CDTb's receptor binding domain 1 (RBD1). Through this effort nine fragments and two novel binding pockets were identified. 2D [¹H, ¹⁵N]-HSQC spectra were collected using nuclear magnetic resonance (NMR) spectroscopy and analyzed using CCPNMR to monitor residue perturbation. The fragments identified will be used in Site Identification by Ligand Competitive Saturation (SILCS) to develop high affinity compounds for the binding sites.

Kate Goundry
University of Virginia
Mentor: Dr. Stuart S. Martin

Tumorigenic Cells Evade Apoptosis in Free Floating Environments

Over the last several decades, breast cancer research has brought about major innovations in treatment and improvements in long term patient survival. However, the presence of metastatic disease still carries a poor prognosis and a variety of complications. The metastatic potential of a tumor is determined in part by the cells' ability to survive traveling to distant sites in the body. Transport typically occurs in the bloodstream and lymphatic vessels where cells exist in a free floating environment, detached from their surroundings. Most nontumorigenic cells, including epithelial cells like the MCF10A cell line do not tolerate detachment and trigger apoptosis. Tumor cells with high metastatic potential, like MB-MDA-436 cells, employ mechanisms and alternate regulation of pathways to allow cell survival in an otherwise unfavorable environment. We compared the survival of detached tumor cells with the survival of nontumorigenic epithelial cells. We have explored two different methods of simulating cell detachment and a free floating environment. We first modeled the prevention of attachment by treating cells with latrunculin A (LA), an actin depolymerase. Through immunofluorescent imaging, we demonstrated that LA destroyed the actin fibers and noticed that there were higher levels of nuclear condensation, indicating apoptosis in treated epithelial cells. Similar actin depolymerization was noted in triple negative breast cancer cells. However, lower levels of apoptosis were seen in this cell population. A free floating environment was also simulated by placing cells in low attach 96 well plates. Apoptosis was measured through propidium iodide staining at 24 hour intervals. In this assay, there were significantly higher levels of apoptosis in the human epithelial cell population than in the MB-MDA-436 population, signifying that the 436 cancer cell line is better equipped to survive in the free floating environment of the bloodstream.

Sona Kocinsky
Tufts University
Mentor: Dr. Charles Hong

Surveying Cancer Type Susceptibility to Ferroptotic Death Via GPR68 Inhibition

GPR68, a G-protein coupled receptor, has recently emerged as a promising therapeutic target for cancer treatment. GPR68 functions as a proton sensor and is overactivated in the acidic tumor microenvironment (TME), promoting increased tumor proliferation, migration, and survival. The expression of GPR68 is known to be highly upregulated in many cancer types, such as glioblastomas, further substantiating it as a potential therapeutic target. The Hong Lab has discovered a novel small molecule, ogremorphin (OGM), that specifically inhibits GPR68. OGM inhibition of GPR68 induces cell death in multiple glioblastoma cell lines via ferroptosis, a newly discovered form of programmed cell death. Considering the elevated expression levels in many cancer cell lines, GPR68 may serve as a mechanism by which these malignant cells thrive in a proton-rich tumor milieu and resist ferroptosis-mediated cell death. Therefore, we hypothesize that GPR68 expression levels directly correlate with sensitivity to OGM-induced ferroptosis and cancer cell survivability. The investigation proceeded on two fronts: assessing OGM sensitivity using cell viability assays and analyzing GPR68 expression with real-time quantitative reverse transcription PCR (qRT-PCR). Both were performed on an array of cell types including immortalized cancers and primary fibroblasts. First, cell death levels were quantitated in response to varied OGM doses using Cell Titer Blue and Cell Titer Glo. In parallel, cell types were analyzed for GPR68 expression levels utilizing qRT-PCR. The GPR68 expression and cell death data were evaluated in tandem to determine if a correlation exists between GPR68, ferroptosis, and cancer cell survival. An understanding of this relationship is crucial to deciphering factors that drive ferroptosis resistance in cancer cells through overexpression of GPR68. This will enable identification of cancer types with the greatest potential for therapeutic benefit with OGM, while also serving to advance knowledge of OGM, ferroptosis, and GPR68. Future research may investigate OGM in vivo for tolerance, safety, and efficacy.

Corinne Martin
University of Maryland College Park
Mentor: Dr. Alexandros Pouloupoulos

Investigating a Mechanistic Link Between Neuronal Axon Projection and Brain Cancer Progression

The formation of the brain's intricate circuitry is driven by the extension of neuronal axons from their origin to specific targets in distant regions, forming networks of synaptic connections. The growth cone of the axon is the director of this movement, and is guided to its proper target through interactions with guidance cues, as well as through the gene expression of the growth cone itself. Here we investigate the idea that genes involved in prompting the extension and migration of axons may play a similar role in stimulating the growth and spread of brain cancer, which is projected to claim the lives of 18,200 American adults this year alone. We used the cancer genomics database cBioPortal to investigate how survival rates of brain cancer patients correlate to the expression of mutations in genes implicated in growth cone movement. One such gene is RPTOR, a subunit of an mTOR protein complex (mTORC1) with a well-established role in cell proliferation and cancer growth. We found that patients who expressed one or more RPTOR mutations had a median survival nearly 50 months longer than that of patients expressing the wild-type gene, leading to the hypothesis that the disruption of this gene may reduce the functionality of mTORC1, decreasing cancer progression and similarly hindering axon projection in the developing brain. We selected six of the patient-specific RPTOR mutations documented on cBioPortal and developed constructs for knock-in using Prime Editing. In tandem, we also developed guides for CRISPR/Cas9 knockdown and knockout of RPTOR, in order to approximate the effects of the mutations with a strategy more conducive to visualization *in vivo*. N2A glioblastoma cells were transfected with each of these constructs in order to manipulate RPTOR expression *in vitro*, and were then assayed for mTOR activity and cell proliferation. The knockdown guide was employed *in vivo* in a mouse model via *in utero* electroporation, in order to visualize the impacts on axon projection. From this approach, we expect to see that edited neurons will display off-target or incomplete connections, supporting a correlation between axon projection and cancer progression.

Eva Gubitz-Hess
Vassar College
Mentor: Dr. Alexandros Pouloupoulos

Investigating Optimal Expression Strategies of CRISPR Agents for Targeted Neural Somatic Genome Editing

Our lab utilizes CRISPR/Cas9 genome editing technology to manipulate risk genes for neurological disorders in mice to better understand their effects on brain circuitry. Through a system of floxed fluorophores and Cre recombinase, we are able to differentiate between control and gene-edited neurons. While CRISPR is a powerful gene editing technology, we have observed that CAG-induced Cre/Cas9 expression in neurons disrupts their normal development. The goal of my project is to find ways to introduce Cre and Cas9 to neurons without causing off-target effects on their development and migration. Using the Golden Gate Assembly cloning method, Cre/Cas9 expression vectors were constructed utilizing CMV, HEF1- α , and PGK promoters to compare their effects on neuronal development. These plasmids will be introduced to fetal mouse brains through *in utero* electroporation in the absence of RNA guides. This will allow me to observe the effects of Cre/Cas9 expression in neurons without gene editing. I will image the mouse brains using fluorescence microscopy for histological analysis. I hypothesize that reducing both the level and timespan of Cre/Cas9 expression will help minimize any off-target effects on neuronal development. It is unclear whether either the level or duration of Cre/Cas9 expression contributes more to off-target effects. This project may help us find ways to optimize the use of CRISPR technology in neural somatic genome editing, which has become an increasingly important research tool in the field of neurological disease.

Eberechi Mbadugha
University of Maryland, College Park
Mentor: Dr. Silvia Montaner

Amphiregulin Promotes Head and Neck Cancer Cell Proliferation Through an ANGPTL4-Dependent Pathway

Recent evidence has shown that human angiopoietin-like (ANGPTL) proteins play a critical role in the initiation, growth, and dissemination of solid tumors. In particular, ANGPTL4 has been seen to be upregulated in a variety of cancers and to correlate with poor prognosis and 5-year survival percentages. This has invigorated the research on ANGPTL4's therapeutic potential for this disease. However, the molecular mechanisms by which ANGPTL4 plays its role in cancer remain unclear. It has recently been discovered that ANGPTL4 plays an important role in the development of head and neck squamous cell carcinoma (HNSCC), which is one of the ten deadliest cancers in the US and around the world. In this study, we investigated the extracellular signals that upregulate ANGPTL4 expression and its downstream pathways in normal and dysplastic human oral keratinocytes as well as human HNSCC cells. We also investigated whether ANGPTL4 regulates the proliferation of these cell lines. We did this by studying the role of amphiregulin (AREG) and epithelial growth factor (EGF) in oral SCC proliferation and in ANGPTL4 expression. We also studied the signaling pathways induced by ANGPTL4 in HNSCC. The aforementioned cell lines are immortalized human-derived normal oral keratinocytes (NOK), human-derived dysplastic oral keratinocytes (DOK) and human-derived malignant HNSCC cell lines HN13 (tongue- T2N2M0), HN6 (base of tongue - T3N2M0), HN4 (base of tongue - T4N1M0), HN12 (lymph node- T4N1M0), and CAL27 (tongue). Procedures include time course experiments of crystal violet assay, MTT Assay, CRISPR induced ANGPTL4 KO, and cell culturing. We saw an increased ANGPTL4 in presence of EGF and AREG, as well as increased tumor cell proliferation. Future directions include confirming our observations in vitro using HNSCC animal models.

Collin Bast
University of Maryland, College Park
Dr. Eli Bar

Optimizing Immunocytochemistry Staining Protocol for the Co-Detection of Nuclear and Plasma Membrane Proteins

Glioma stem cells and cancer stem cells in several other tumor types residing outside the central nervous system promote tumor progression and therapeutic resistance. The epidermal growth factor receptor (EGFR) encodes plasma-membrane proteins that promote cancer cell proliferation and rapid tumor growth of glioblastoma (GBM) and other cancers. Our working hypothesis is that EGFR amplification, which is a common genetic feature (up to 55% of all GBM), promotes drug resistance. Since EGFR expression is mosaic, determining if cells overexpressing EGFR represent a drug-resistant subpopulation within GBM cultures and tumors would be beneficial. Immunocytochemistry staining utilizes highly-specific binding molecules in order to detect and display various molecules in biological specimens. This technique was utilized for the staining of EGFR and γ H2AX proteins. γ H2AX binds to double-stranded DNA damage in the nucleus, and when stained can be used to model the amount of degradation inflicted by cancer therapies. A protocol for γ H2AX staining was previously established in the Bar laboratory to model the efficacy of GBM treatments. This study aimed to develop optimal staining conditions for the detection and quantification of EGFR expression and the DNA damage marker γ H2AX. Successful staining procedures were established for the separate staining of EGFR and γ H2AX proteins. Favorable conditions were recorded for the primary and secondary antibody dilutions, as well as the fixing solution. However, further study should be conducted to produce the most effective permeabilization methodology. The optimization of this protocol will play a key role in quantifying DNA damage in tumor cells expressing varied levels of EGFR. Following the completion of a staining procedure, an algorithm may be developed for the quantification of γ H2AX punctations and level of EGFR expression. Coupling the γ H2AX staining process with EGFR ultimately allows for a relationship to be drawn between the efficacy of treatment and expression of EGFR.

Kyle Gunzy
Loyola University Maryland
Mentor: Dr. Lishan Su

Development of BDCA2-targeting antibody using yeast surface display human antibody library

Plasmacytoid dendritic cells (PDCs) play an important role within the innate and adaptive immune systems. PDCs release interferons, which are proteins involved in the body's natural defense against viruses and diseases. Blood DC antigen 2 (BDCA2) is a human PDC-specific antigen that inhibits interferon release. Preliminary studies have discovered that PDCs can cause pathogenic effects within inflammatory tissues that lead to autoimmunity, cancer, and chronic viral infection. Using yeast surface display, we will be isolating antibodies with a high affinity for binding BDCA2. The yeast surface display experiments require a three-day protocol, which include BDCA2 biotinylation, library expansion of the yeast cells, library induction, and library selection using MACS beads. Multiple rounds of selection are conducted during the library selection process in which presort, two rounds of negative selection, and positive selection samples are collected. The cells within these various samples are then placed on an agar plate and counted after two days of incubation. Finally, the use of flow cytometry will be employed to analyze any antibody clones obtained from our experiment through multiple rounds of selection. These antibody clones will then be used to develop a drug that can target the BDCA2 antigen and prohibit PDCs from interferon release, which will optimize the body's immune system and defense against autoimmunity, cancer, and infection.

Brianna Dewan
Stevenson University
Mentors: Dr. Ryan Pearson and Nhu Truong

The Role of Nanoparticle Formulation and Purification Parameters on Macrophage Drug Delivery

The enhanced permeability and retention (EPR) effect has become a key component in cancer nanomedicine development to enhance the treatment of solid tumors. The EPR effect describes the tendency of smaller particles, such as nanoparticles (NPs), to accumulate in vascularized tumor microenvironments (TME) compared to normal tissues. Present in these TMEs are tumor-associated macrophages (TAMs) which promote tumor progression. Targeting TAMs through the use of NP-based drug delivery systems has the potential to mitigate tumor development. The physicochemical properties of NPs can be modified to influence the cellular uptake by TAMs. Preliminary data establishing the use of a microfluidics device to generate various NP formulations demonstrated that surfactant type and flow rate ratio (FRR) used to synthesize the NPs changes its physicochemical properties. It was shown that the surfactant type altered the NP's surface chemistry and increasing FRR led to decreased size. For this study, poly(lactic acid) PLA was used as the core polymer and poly(glutamic acid) (PGA) and poly(vinyl-alcohol) (PVA) were used as the surfactants to modify the NP surface potential. The NPs were synthesized using a high-throughput microfluidics-based method, and then purified by a tangential flow filtration (TFF) system, which was optimized at specified conditions. The physicochemical properties of the NPs were characterized via dynamic light scattering (DLS) analyzing for particle size, polydispersity index, and zeta potential. The aim of this study was to assess the ability of four NP designs that contain a fluorophore (Cyanine 5.5) to be taken up by bone-marrow derived macrophages (BMDMs). To assess the cellular uptake of the NPs into BMDMs, BMDMs were treated with the fluorophore NPs and flow cytometry was used to assess for fluorophore positive cell interactions with the fluorescent NPs. The present methodology to formulate well-defined NP-based drug delivery systems sets the stage for future development of cancer-targeted nano-immunotherapies.

Katie Donnelly
University of Kansas
Mentor: Dr. Nevil Singh

Targeting cytokine immunotherapy to the tumor using IL-12 subunits

IL-12 is a heterodimeric cytokine that has shown to be effective in fighting tumors, due to its ability to convert tumor-promoting T cell responses to inflammatory anti-tumor activity. In clinical trials, administration of intravenous IL-12 led to high levels of inflammation and even patient mortality – due to systematic immune activation ^[1]. Thus, another method of creating IL-12 specifically at the tumor microenvironment site is needed. It has been shown previously in our lab that the individual subunits that make up IL-12 (p40 and p35), can dimerize outside of the cell to form IL-12 and induce INF- γ production. ^[2] The p40 subunit, which is inert by itself, will thus allow IL-12 to be made specifically where the p35 subunit is released. The p35 current secretion pathway is unknown, however; the subunit has been detected after harsh freeze-thawing that resulted in cell death ^[3]. Based on this, we hypothesized that IL-12p40 can combine with IL-12p35 released during necrosis by non-immune cells. In a tumor, necrosis within the tumor microenvironment could be extensive – due to rapid cell growth of cells, which create a hypoxic environment. ^[4] Based on our hypothesis, we tested two aims in this proposal. First, we examined how dosing with p40 will drive anti-tumor immunity. Second, we are developing a new approach to monitor how and when p35 can be released so it can be manipulated for concentrated IL-12 production in the tumor microenvironment. Taken together, we expect these results to lay out a new paradigm for immunotherapy and further understand the opportunities for fine tuning this towards clinical translation.

Erika Carmody
Fordham University
Mentor: Dr. Nevil Singh

Improving CAR-T therapy by targeting signaling pathways regulating cell survival

The adoptive transfer of T cells and CAR T cells can be used to treat certain cancers. CAR T cells are genetically modified T cells that have been engineered to specifically detect surface markers of certain cancer cells. While highly effective when first given, these adoptive T cell therapies are often not sustained, possibly due to T cell death, inactivation, or loss of function. As a result, tumors can relapse and the repeated infusion of T cell therapy, specifically CAR T cell therapy, is financially and physically costly for the patient. Therefore, genetic manipulation of CAR-T cells to improve their survival and function would be quite impactful in the clinic. Our lab had previously shown that a T cell transmembrane protein, CD5, increases the levels of the transcription factor NF κ B and potentially enhances cell survival. CD5 is a complex protein with multiple intracellular domains, some of which have negative-regulatory roles. We found that a part of the intracellular portion of the CD5 protein, called CD5-Min, was sufficient for the increase in NF κ B. We therefore hypothesized that overexpressing CD5 Min would increase NF κ B, thus increasing T cell survival, in both *in-vivo* and *in-vitro* models. We transduced a T cell line (BW5147) and validated that the CD5 Min construct led to an increase in I κ B through intracellular staining and flow cytometry analysis. We then injected mice with primary T cells transduced with CD5 New Min and the control, and we are currently in the process of exploring if I κ B is also increased in the primary T cells after seven days *in-vivo* through analysis by flow cytometry. We would next look directly at the survival of primary T cells during adoptive T cell therapy to confirm that the increase in I κ B directly correlates with increased T cell survival of those expressing CD5 Min. Additionally, the experiment would be repeated with CAR T cells transduced with CD5 Min. Taken together, our experiments can identify a new approach to improving tumor immunotherapy.

Jane Kimani
Indiana University
Mentor: Dr. Tonya Webb

Natural killer T cell Responses to Lung Cancer

Natural killer T (NKT) cells are a subset of T cells that are activated by lipid antigens presented in the context of CD1d molecules. Following activation, NKT cells rapidly secrete cytokines and can kill cancer cells. Therapies targeting NKT cells in cancer patients with highly functional NKT cells can result in a significant increase in survival; however, many cancer patients have a reduction in NKT cell number and function. Our lab has recently developed a nanotechnology platform, using CD1d-based artificial antigen presenting cells (aAPC), which can be used to activate and expand NKT cells. Thus, we sought to test the hypothesis that activation with aAPCs can enhance NKT cell responses to lung cancer. To investigate NKT cell responses to lung cancer, NKT cells were cocultured with a panel of lung cell lines in the presence and absence of the NKT cell agonist, α -Galactosylceramide (α -GalCer). We found that the lung cancer cell lines did not activate NKT cells, even in the presence of α -GalCer. Next, we assessed NKT cell activation following stimulation with different formulations of CD1d-based aAPC. It was found that aAPC expressing anti-CD44mAb were potent activators of type I NKT cells. Future studies will investigate the therapeutic efficacy of combinational approaches targeting CD44 and NKT cells for the treatment of lung cancer.

Aliqua Christensen
Spelman College
Mentor: Tonya J. Webb

Monoglyceride Lipase Regulates Lymphocyte Activation

Monoglyceride Lipase (MGL) is a serine hydrolase that catalyzes the conversion of monoacylglycerides to free fatty acids and glycerol and has recently been shown to play a role in tumorigenesis. MGL deficient (MGL KO) mice were developed to investigate the role that MGL plays in cancer development. It was found that MGL KO mice exhibit splenomegaly and develop tumors in organs such as the spleen, liver, and lung, at a much high rate than wildtype littermate control (MGL WT) mice. Therefore, we sought to test the hypothesis that MGL KO mice have a defect in cancer immune surveillance due to impaired immune cell function. To investigate the mechanisms by which the loss of MGL results in defective antitumor immune responses, we examined splenic lymphocyte activation following stimulation with anti-CD3/28 microbeads or PMA/ionomycin. In these studies, spleens were harvested from sex-matched, age-matched MGL WT and KO mice and single cell suspensions were generated. The cells were stimulated for 48hrs and cytokine ELISAs were performed to assess lymphocyte activation. We found that splenocytes from MGL WT and KO secreted different levels of IL-2 and IFN- γ following activation. These data suggest that MGL plays a pivotal role in regulating immune cell activation. Subsequent studies will focus on examining the kinetics of T cell activation and investigating specific signaling pathways that may be impaired in MGL deficient mice.

Pranav Majeti
University of Maryland College Park
Mentors: Drs. Matthew Frieman and Stuart Weston

Assessing the SARS-CoV-2 genome for slow-growth phenotype in *Saccharomyces cerevisiae* for the identification of potential therapeutic targets

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, which is the causative agent for COVID-19, has affected over 560 million people worldwide. Despite the rapid output of vaccines, there is still a large infectivity rate of the virus. This rapid emergence of the COVID-19 pandemic has increased the need for 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 regulation of viral genomes, while accessory proteins function as virulence factors involved in pathogenesis, making these proteins potential therapeutic targets. Yeast, *Saccharomyces cerevisiae*, has been widely used to understand eukaryotic cell biology and can be used to understand interactions between viruses and eukaryotic cells. Previously, our lab found that you can express proteins from viruses, such as Middle East respiratory syndrome coronavirus and the Influenza A Virus, in yeast which cause phenotypic effects, such as slow growth. Using a similar approach, we want to utilize this slow growth phenotype for proteins for SARS-CoV-2. Therefore, we propose to investigate the SARSCoV-2 proteome to identify proteins that cause a slow growth phenotype when expressed in yeast, allowing the identification of genetic suppressors. Nonstructural genes of the SARS-CoV-2 genome were cloned into a galactose inducible vector, possessing a C-terminal green fluorescent protein tag, and then transformed into yeast. Single yeast cell colonies were picked and grown to achieve stationary phase in CAA media containing raffinose. Then yeast cultures were diluted in media containing galactose to induce gene expression, and growth phenotypes were analyzed by measuring optical density. To further discern growth phenotype, drop cultures of yeast were performed on a petri dishes with glucose and galactose to analyze slow growth phenotype by utilizing the galactose-inducible vector. From these studies, we hope to gain a better understanding on how SARS-CoV-2 proteins interact with eukaryotic cells and potentially develop antiviral therapies for highly pathogenic coronaviruses.

Darielle Runner-Draper
Loyola University Maryland
Mentors: Dr. Alonso Heredia

Effects of Altering FC Binding Region of HIV Antibody

In the United States 1.2 million people have HIV (HIV, 2022), HIV can lead to AIDS and many other opportunistic infections. Currently the standard of care to treat HIV is called antiretroviral therapy (ART) (NIH, 2021), which causes many negative side effects with the digestive tract, skin and neurological system (Chen et. al, 2013). The FC region of an antibody is the bottom or tail portion, it is important for binding to cell surfaces and activation of the immune system. A portion of the FC region that is directly responsible for FcRn binding also plays a role in the recycling of antibodies which increases the half-life (Roopenian & Akilesh, 2007). Modifications on the Fc portion of antibodies for respiratory syncytial virus increased the half-life from about 20 days to greater than 60 days (Robbie et al., 2013). In this study we modified the monoclonal HIV antibodies in the Fc binding region which we expect to increase the binding to FcRn region increasing the half-life. Humanized mice with either wild type, modified 1 or modified 2 antibody. Blood samples were taken 10 times in the duration of 8 weeks and the plasma was extracted. The concentration of the antibody was determined based on the color change using a process called enzyme-linked immunosorbent assay (ELISA). We determined that the modification to "Modified 1" antibody increased the half-life compared to the "Wild Type" or "Modified 2" antibodies. Future studies should determine if the modifications have any other physical effects to the Fc region that could affect binding.

James Hsia
Howard University
Mentor: Dr. Daria A. Gaykalova

Detection of HPV Integration Status in Head and Neck Cancer through PCR techniques and Characterization of Human-Viral Junctions in scRNAseq data

Head and Neck Squamous Cell Carcinomas (HNSCCs) are the sixth most common type of cancer, with 890,000 new cases reported and rates projected to increase significantly. HPV-associated HNSCC rates are also expected to increase by 30% by the year 2030 and already make up a significant proportion (at least 44%) of cases of head and neck cancers, especially in the industrialized nations of Europe and the United States. HPV-positive HNSCC exhibits behavior distinct from HPV-negative HNSCC, and is usually caused by the strain HPV 16. In HPV-positive HNSCC, oncogenesis commonly occurs through expression of oncogenes E6 and E7 in the HPV genome. The HPV genome can also be integrated into the host genome from its original form as a viral episome. However, detecting the integration status of HPV in tumor cells is strenuous, requiring the use of whole genome sequencing (WGS) or RNAseq. We plan to develop and test a method to determine the integration status of HPV by using long range PCR with newly designed primers. We expect that it will yield sequences of distinctive lengths when applied to HPV-positive HNSCCs due to the variable lengths of PCR products that will be synthesized depending on whether the virus is present in episomal form or integrated form in the sample. Several cell lines with known HPV integration status will be used to test this method. Gene expression of E6 and E7 is mediated by the HPV E2 protein, but its gene and that of protein E5 are both disrupted by the process of integration into the host genome. Therefore, the ratios comparing E2 protein expression and E6 and E7 expression can be indicative of HPV integration status. qRT-PCR and Western blotting were used to study this in the same cell lines used for PCR. Integration sites are of particular interest because of their possible heterogeneity between cells in HNSCC tumors. We plan to locate and possibly define these human-viral junctions in single cell RNAseq (scRNAseq) data using bioinformatic tools such as STARsolo and FastViFi. We hope that in the future this PCR technique can be tested on patient samples and human-viral junctions can be further studied to provide therapeutic targets.

Sophie Liu
Johns Hopkins University
Mentor: Dr. Michal Zalzman

ZSCAN4 knockout reduces in vitro cancer stemness in head and neck squamous cell carcinomas

Cancer stem cells (CSCs) are a subpopulation of tumor cells that can guide tumor initiation and metastasis. Their ability to self-renew and differentiate into heterogeneous lineages of tumor cells render them less sensitive to traditional modes of cytotoxic therapy like chemotherapy and radiation. Our lab has previously demonstrated that the Zinc Finger and SCAN domain containing 4 (ZSCAN4) gene contributes to cancer stem cell maintenance. Through histone acetylation, ZSCAN4 induction opens CSC-associated chromatin and thereby promotes an increased expression of stemness genes. In the present study, we assessed the stemness properties and proliferative capacity of Tu167 cells using *in vitro* techniques. Here, we demonstrate the contribution of ZSCAN4 in head and neck squamous cell carcinomas (HNSCCs) by generating CRISPR/Cas9-mediated ZSCAN4 knockouts (KO) in HNSCC lines. Wild type (WT) and Cas9 without sgRNA were used as controls. We validated the resultant bulk KO lines by sanger sequencing and subsequent ICE analysis. We then characterized the cell line in regards to proliferation, stemness and CSC gene expression, *in vitro* tumorsphere formation capacity, and resistance to clinically relevant chemotherapeutics. Our results demonstrate that ZSCAN4 KO leads to a reduction in potency and CSC gene expression, and reduced *in vitro* tumorsphere formation, without significant changes in proliferation rate or drug resistance. This work further confirms ZSCAN4's role in maintaining cancer stemness, making it a promising target for future CSC-depleting cancer therapies.

Catie Corolla
Loyola University Maryland
Mentor: Dr. Paul Shapiro

Targeted inhibition of extracellular signal-regulated kinase 1/2 functions to inhibit cancer cells

Mutations in the Ras-Raf-MEK-ERK signaling pathway are found in many human cancers, leading to increased cell proliferation. Therefore, this pathway has been of interest in treating cancer, specifically by targeting extracellular signal-regulated kinase 1/2 (ERK1/2). Current drugs that inhibit ERK1/2 are ATP-competitive, thus blocking all ERK1/2 function. However, ERK1/2 has hundreds of substrates, not all of which are oncogenic, and patients develop resistance to these drugs relatively quickly. A novel small molecule inhibitor, SF-3-030, was previously developed to more selectively inhibit ERK1/2 at a specific substrate binding site so that only oncogenic substrates of ERK1/2 are inhibited, but other functions remain intact (Samadani 2015). Here we examine the effects of SF-3-030 on ERK1/2 and downstream activator-protein-1 (AP-1) proteins, which are a complex of transcription factors that regulate genes involved in cell proliferation. A HeLa cell line was treated with SF-3-030, isolated into nuclear and cytosolic fractions, and immunoblotted to see the effect of SF-3-030 on ERK1/2 and AP-1 activity. In this cell line, we did not find conclusive evidence that SF-3-030 inhibits ERK1/2 nuclear translocation or the phosphorylation of AP-1 proteins. Future studies should continue to examine the role of SF-3-030 on the inhibition of AP-1 proteins, as well as examining the cytosolic targets of ERK1/2 and any compensatory effects of SF-3-030 on other pathways. In addition, using many cell lines, especially those that have ERK1/2 pathway mutations, would be helpful to compare the effectiveness of SF-3-030 on different cancer types. By elucidating the mechanism of SF-3-030's selective inhibition of ERK1/2, we hope to improve on current ERK1/2 inhibitors as a more effective cancer therapy in patients with ERK1/2 pathway mutations.

Christina Cummins
Pennsylvania State University (Main Campus)
Mentor: Dr. Paul Shapiro

Targeted Inhibition of ERK 1/2 Functions in Large B cell Lymphoma

The extracellular-signal-regulated kinases (ERK1/2) function in the RAS-RAF-MEK-ERK pathway to regulate a variety of cellular activities. However, activating mutations in the RASRAF-MEK-ERK pathway promote cancer cell proliferation. Thus, ERK1/2 is a potential anticancer drug target. The current ERK1/2 inhibitors target the ATP binding site, block all enzyme activity, cause unwanted toxicity and drug resistance. This study explores a novel function selective ERK1/2 inhibitor, SF-3-030, that was designed to target substrates that cause cancer cell proliferation. Working with the HBL-1 DLBCL cell line, we hypothesized that SF-3-030 will target the substrate binding site on ERK1/2 to effectively inhibit its translocation into the nucleus, therefore inhibiting transcription that induces cancer cell proliferation. This cell line posed as an interesting subject, because of its aggressive nature possibly attributed to the upregulation of ERK1/2 activity. Cytoplasm and nuclear protein fractions were generated from stimulated HBL-1 cells immunoblots were done to evaluate total and active ERK1/2 levels in the cell fractions. Quantitative analysis suggested that SF-3-030 had no effect on inhibiting ERK1/2 nuclear translocation. However, examination of ERK1/2 substrates indicated that SF-3-030 did inhibit phosphorylation of the immediate early gene, c-Fos, which is an important transcription factor that promotes cancer cell proliferation.

Rohaan Mahmud
University of Maryland College Park
Mentor: Dr. Gerald Wilson

Identifying Tumor-suppressive Mechanisms of the RNA-binding Protein TTP in Breast Cancer Cell Models

Tristetraprolin (TTP) is an AU-rich element-binding protein that regulates the stability of many mRNAs that encode proteins involved in the initiation and progression of cancer. Previous studies have shown that limiting TTP levels can increase tumor grade and negative patient outcomes while also showing that restoring TTP expression in some cancer cell models can slow proliferation and tumor growth. However, prior research in aggressive breast cancer cell models also showed consistent levels of known TTP mRNA substrates (like VEGF) regardless of whether TTP was expressed or not, and that re-introduction of TTP did not accelerate decay of known TTP-targeted transcripts. These data support the hypothesis that the tumor suppressive activities of TTP may function through an alternative pathway that does not involve RNA binding and/or mRNA destabilization. To discover potentially novel mediators of TTP function, RNA-Seq was used to identify RNAs that were differentially expressed when TTP was reintroduced into MDA-MB-231 cells. Candidates were prioritized for those with known roles in control of cell proliferation and included CCND2, WT1, RASSF2, and TLE3. To evaluate whether these factors were regulated by TTP in multiple breast cancer cell models, the effect of TTP expression on mRNA and protein levels were measured across three aggressive breast cancer cell lines: MDA-MB-231, MDA-MB-436, and BT-549. At both the mRNA and protein levels, the putative tumor suppressor protein Ras association domain family member 2 (RASSF2) was significantly induced when TTP was overexpressed in MDA-MB-231 and -436 cells, suggesting that it might play a role in TTP's antiproliferative activity in these cell lines. With RASSF2 now a potential target, overexpression and knockdown trials are underway to determine whether proliferation decreases in control cells when RASSF2 is introduced or increases in TTP-expressing cells when RASSF2 is depleted. If RASSF2 is shown to be critical for the TTP-dependent antiproliferative phenotype, subsequent experiments will determine whether TTP regulates RASSF2 by transcriptional and/or posttranscriptional mechanisms. Together, these data may identify RASSF2 as a critical mediator of TTP's antiproliferative activity in breast cancer models.

Patrick McGinnis
Loyola University Maryland
Mentor: Dr. Gerald Wilson

Roles of AUF1 Protein Domains in RNA Binding and Structural Remodeling

AUF1 is an RNA-binding protein that binds AU-rich elements (AREs) and functions to regulate gene expression through modulating mRNA degradation, stabilization, translational efficiency, and through interaction with both miRNAs and lncRNAs. AUF1 has an established role in cancer as many of its target transcripts encode products that regulate pro- and anti-oncogenic processes. Elevated AUF1 levels contribute to a variety of cancers by promoting cell proliferation and invasion, including in breast and thyroid cancers, and is related to the malignancy of hepatic cancer. Identifying the mechanisms by which AUF1 recognizes and regulates RNA substrates may provide novel targets for therapeutic development.

Among AUF1's known biological functions are the binding and structural remodeling of various RNA substrates. However, the mechanism(s) by which AUF1 performs these tasks remains largely unknown. My project focuses on identifying protein domains on the p37 isoform of AUF1 that are required for RNA binding and conformational remodeling. I am utilizing protein domain mutants to interrogate the contribution of each domain to RNA binding affinity using fluorescence anisotropy, and RNA remodeling using a Fluorescence Resonance Energy Transfer (FRET)-based assay.

All isoforms of AUF1 contain 2 tandem RNA Recognition Motifs (RRMs), and previous experiments have shown that both are required for high-affinity RNA binding. However, strong RNA binding also requires C-terminal sequences although the mechanism by which they stabilize ribonucleoprotein complex structure is unknown. As AUF1 functions to both bind and remodel RNA substrates, we expect the C-terminus to have a similar contributive function to local RNA remodeling, more so than either the RRM or the N-terminus flanking domain. Establishing the protein domain(s) required and most important for AUF1's RNA remodeling activity is necessary to begin understanding how an inhibitor or therapeutic target could function in modulating AUF1 activity and roles in cancer proliferation and progression.

Danielle Jones
Georgia Institute of Technology
Mentor: Dr. Allison Scott

The Impact of Bacteria and Metabolite Quantitation in Predicting Dental Caries

Dental caries, also known as cavities or tooth decay, is the most widespread non-communicable disease worldwide and one of the most common chronic diseases in pediatric patients¹. If left untreated, dental caries can lead to tooth loss, infections, abscesses under the gums, and even sepsis in severe cases. Early caries are difficult to detect, however, which can result in delayed or inadequate treatment. The nature of molar contact is one proposed factor influencing the early development of dental caries. Previous studies have proposed a classification system for the orientation of primary molar contacts based on the appearance of the contact surface, described as Open, X-shaped, I-shaped, or S-shaped, (OXIS classification). The OXIS classifiers correlated these orientations to dental caries formation, and it was determined that I and S classifications correlate to higher rates of subsequent dental caries. However, in order to use OXIS classification to identify patients that are predisposed to dental caries, it is necessary to identify other measurable biomarkers associated with the OXIS type. Here, we seek to determine the metabolite and bacterial profiles of dental plaque to determine whether the correlation between primary molar orientation and dental caries is independent of other factors (e.g. oral microbial burden). We hypothesize that I and S-type primary molar contacts will have a higher proportion of cariogenic bacterial species and metabolites of an acidified microenvironment which is responsible for an increased number of dental caries. To successfully identify a metabolic and microbiological "signature" of dental plaques during the progression of childhood dental caries, we performed an inline metabolite and DNA extraction followed by targeted metabolomics for organic acids and select bacterial quantitation using qPCR with a "spike" DNA construct. This was then related back to the OXIS classification of the sample to potentially discover a relationship between the contents of dental plaque and primary molar orientation. These results were used to determine an optimized workflow for plaque samples from a clinical cohort.

Rebecca Oluwasanmi
University of Maryland College Park
Mentor: Dr. Karen Scanlon

***Bordetella pertussis*-induced IDO responses are age-dependent**

Bordetella pertussis causes a dangerous, sometimes deadly infection in infants and there are currently no remedial solutions. *B. pertussis* is age dependent and causes acute manifestations such as seizures, pneumonia, and pulmonary hypertension that are exclusive to infants and not seen at an older age. We investigated the age-dependent expression and regulation of an immunosuppressive molecule called Indoleamine-2,3-dioxygenase (IDO). IDO is an enzyme that is encoded by the gene IDO1 in mammals. IDO plays a role in many diseases, for example, cancer and respiratory syncytial virus (RSV) infection. We also examined the correlation between IDO expression and the regulation of inflammation, by measuring interferon-gamma (IFN- γ) and interleukin 10 (IL-10) levels. We hypothesized that the expression of IDO in the lungs is age-dependent, with significant upregulation in early life, and in infants, IDO functions limit inflammation but consequently promote bacterial outgrowth and severe pertussis manifestations. To test this hypothesis, IDO-expressing (C57BL/6) and IDO-deficient (IDO Knockout) infant (postnatal day 7) and adult (6-8 weeks) mice were infected with *B. pertussis*. Their lung tissue was harvested 3 days post-inoculation. The lungs were then processed for transcript analysis and for protein analysis. Our results displayed that our hypothesis stating IDO expression levels are age-dependent was true. The results also showed that IDO expression correlates with reduced IFN- γ levels. This is consistent with the immunosuppressive function of IDO. We found out that IL-10 is detectable in the lungs of *B. pertussis*-infected mice. Surprisingly, IL-10 concentration was highest in IDO-expressing adult mice.

Tyonna Tyson
Hampton University
Mentor: Dr. Julie Dunning Hotopp

Developing a Bioinformatic Tool for Improved Prokaryotic Differential Expression Analyses

An alarming number of human pathogenic bacteria are acquiring resistance to antibiotics which presents a challenge for physicians to effectively treat infections. One method of better understanding the regulatory processes and mechanisms involved in the acquisition of resistance and increased pathogenicity is differential expression analysis. Current prokaryotic differential expression analysis tools, however, can often underestimate or miss critical pathogenic genes because prokaryotic transcriptomic analyses rely heavily on protein coding sequences (CDS) rather than the full transcript. To develop a bioinformatics tool that analyzes transcripts as opposed to only CDS, we will use Oxford Nanopore Technologies (ONT) direct RNA sequencing to obtain sequences to identify operons in a highly and a mildly pathogenic *Escherichia coli* using culture conditions that are known to induce differential expression. In order to generate RNA transcription sequencing data, high quality RNA had to first be isolated from bacterial cells harvested in early log phase. We grew *E. coli* strains K12 and E2348/69 in the high nutrient environment of Luria-Bertani (LB) broth along with the low nutrient environment of the Dulbecco's Modification of Eagle's Medium (DMEM). Total RNA was then isolated from the bacterial cultures at an optical density (OD 600) of ~0.50 using the Qiagen RNeasy Protect Bacteria Protect protocol with on-column DNase digestion. RNA quality was assessed using the Agilent TapeStation and Qubit. The RNA integrity numbers (RIN) for all four conditions were ≥ 8.1 and the concentrations ranged from 1.6 to 13 μg . Given the high quality and sufficient quantity of this RNA, it is very likely these transcripts will be successfully sequenced on the ONT MinION R9 flow cell. The resulting sequencing reads can then be used to create an effective bioinformatics tool that will analyze prokaryotic transcripts.