The 2019 Summer Research Symposium of the Nathan Schnaper Intern Program in Translational Cancer Research

25 July 2019
Health Sciences Facility III room 1010

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

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NSIP
Nathan Schnaper Intern Program
in Translational Cancer Research

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

2019 NSIP Research Symposium

Thursday, July 25, 2019
9:00 am to 4:35 pm
HSFIII rm 1010

	Speaker	Mentor
8:30 to 9:00 am	**Breakfast**	
9:00 to 9:15 am	**Bret A. Hassel, PhD** *Director's Welcome*	
9:15 to 9:30 am	**Carina Sclafani** *Identification of Novel Regulators in Human Erythropoiesis in vitro*	Tami Kingsbury
9:30 to 9:45 am	**Sulan Wu** *Molecular and structural characterization of low grade serous ovarian cancer*	Amy Fulton
9:45 to 10:00 am	**Megan Stemberger** *Targeted degradation of oncogenic mRNA targets using an engineered zinc finger ribonuclease*	Gerald Wilson
10:00 to 10:15 am	**Nina Lee** *Roles of specific domains of AUF1 in functional recognition of RNA substrates*	Gerald Wilson
10:15 to 10:30 am	**Kaitlyn Zwerling** *The Identification of Human Proteins, Regulating Influenza Virus Replication*	Matthew Frieman
10:30 to 10:45 am	**Break**	
10:45 to 11:00 am	**Heather Buck** *Restoring Treatment Sensitivity to Resistant Melanoma Cells in Spheroid Culture*	Paul Shapiro
11:00 to 11:15 am	**Alvin Ya** *Characterizing the Changes in Metabolic Gene Expression in Cancers with PI3K Pathway Mutations*	Sui-Seng Tee
11:15 to 11:30 am	**Sherry Fan** *An NMR based fragment screen of heterogeneous ribonucleoprotein A18*	David Weber
11:30am to 11:45pm	**Thomas Hill** *Mammalian Expression of IgNAR-Derived Binding Domains (VNARs) Targeting HER1 and HER3 Cancer Markers*	Helen Dooley

11:45 to 12:00 pm	**Alexandra Greenbaum** *Regulation of chromatin state in cancer stem cells*	Michal Zalzman
12:00 to 12:15 pm	**Lauren Sands** *The role of ANGPTL4 (Angiopoietin-like 4) in head and neck squamous cell carcinoma (HNSCC) metastasis*	Silvia Montaner
12:15 to 1:15 pm	**Lunch with Drs. Lauren Schnaper and David Carolton**	
1:15 to 1:30 pm	**Sophie Nerone** *Targeting Pancreatic Adenocarcinoma Using a Combination of Pegcrisantaspase and the Bcl-2 Inhibitor Venetoclax*	Ashkan Emadi
1:30 to 1:45 pm	**Christina Kratzmeier** *Sequence Validation of the FLT3 F691L Point Mutation in AML Cells*	Rena Lapidus
1:45 to 2:00 pm	**Nicole Illesca** *DNA Double Strand Break Repair Capacity in AML after HDAC Inhibition*	Feyruz Rassool
2:00 to 2:15 pm	**Emilee Stenson** *The role of DNA binding activity of cell cycle checkpoint protein Rad9 in DNA repair*	A-Lien Lu-Chang
2:15 to 2:25 pm	**Break**	
2:25 to 2:40 pm	**Blair Landon** *Development of a blood brain barrier model for screening brain tumor nanotherapeutics*	Anthony Kim, Jeff Winkles, and Graeme Woodworth
2:40 to 2:55 pm	**Darien Campisi** *Development of a safe and effective non-viral gene delivery platform targeted to immune cells*	Ryan Pearson
2:55 to 3:10 pm	**Richa Beher** *Inflammasome Activation Induces Spatial Regulation of Metabolic Enzymes in Tumor Cells*	Greg Szeto
3:10 to 3:25 pm	**Laura Gerrick** *The Role of N191 in acid-catalyzed extraction of 5-carboxylcytosine by Thymine DNA Glycosylase*	Alex Drohat
3:25 to 3:35 pm	**Break**	
3:35 to 3:50 pm	**Mariah Lee** *Human Treg cells express Plexin B1 and Neuropilin 2, two functional receptors for neuroimmune semaphorins*	Achsah Keegan

3:50 to 4:05 pm	**Sara Fioretti** *Brewing up a Storm: Modeling Anti-CD-19 CAR T-cell Adverse Side Effects in Mice*	Nevil Singh
4:05 to 4:20 pm	**Benjamin Atkinson** *Analysis of Granzyme B Expression in a Murine Ex Vivo Model of Bone Marrow Derived MDSC's*	Xuefang Cao
4:20 to 4:35 pm	**Perry Summers** *Elucidating the role of Bcl-xL on CD1d-mediated NKT cell activation*	Tonya Webb

Abstracts

(in speaking order)

Carina Sclafani
Wheaton College
Mentor: Dr. Tami Kingsbury

Identification of Novel Regulators in Human Erythropoiesis in vitro

Development is orchestrated via conserved families of transcription factors controlling cell proliferation, fate specification, and differentiation. Erythropoiesis, the process by which rare hematopoietic stem-progenitor cells produce ~ 200 billion red blood cells (RBCs) per day serves as a paradigm for transcriptional regulation of development. The GATA1 transcription factor, in conjunction with FOG1, is a master regulator of erythropoiesis. Our lab recently discovered that members of the PAX-SIX-EYA-DACH transcriptional network (PSEDN) can stimulate erythropoiesis in a GATA1-dependent manner. Like GATA factors, PSEDN members regulate diverse tissue formation during development across species and inappropriate expression promotes cancer development and progression. GATA and PSEDN members have been studied extensively on their own, but only recently have we demonstrated novel physical and functional interactions between GATA and SIX proteins. The goal of this project is to identify protein domains mediating SIX1/SIX2 interaction with GATA1. Toward this goal, we have generated a series of GATA1 and SIX deletion constructs for use in yeast two-hybrid (Y2H) assays designed to map essential protein interaction domains. Unexpectedly, expression of SIX1-LexA DNA binding domain fusion protein alone conferred robust reporter gene expression, revealing the presence of a transcriptional activation domain (TAD). This is the first demonstration of a TAD within SIX1. Deletion mutant analysis is underway to map the SIX1 TAD. The data obtained from these analyses will be utilized in future experiments aimed at understanding functional consequences of disrupting SIX-GATA interaction on normal and malignant hematopoietic transcriptional programs, growth and differentiation. The ultimate goal of this line of investigation is to determine whether disruption of SIX-GATA interaction could be targeted for therapeutic intervention in cancer, especially since SIX proteins are not expressed in most adult tissues.

Sulan Wu
Oberlin College/California Institute of Technology
Mentor: Dr. Jocelyn Reader and Dr. Amy Fulton

Molecular and structural characterization of low grade serous ovarian cancer

The second most lethal gynecological malignancy, low grade serous ovarian cancer (LGSOC), is a rare and indolent carcinoma that represents less than 10% of serous ovarian carcinomas. However, LGSOC exhibits chemoresistance to standard cytotoxic chemotherapy for ovarian cancer, which is more effective in targeting the fast-growing high grade serous ovarian cancer (HGSOC). Consequently, there is a large, unmet need for novel chemotherapeutics specific to the slow-growing and chemorefractory characteristics of LGSOC. Microtentacles (McTNs), or microtubule-based protrusions of the plasma membrane, are of interest due to their involvement in the metastatic process. We hypothesize that McTNs and other molecular characteristics of LGSOC can serve as useful targets in the development of novel chemotherapeutics. In order to begin to test this hypothesis, data was collected on the type and number of McTNs expressed in three LGSOC cell lines using a cell tethering technology that holds cells in stationary suspension. Once tethered, cell images were captured via confocal microscopy and analyzed. We also performed an analysis of gene expression and protein localization of potential therapeutic targets that were chosen due to their roles in drug response and cellular structure, including EGFR, EP4, HuR, actins, and tubulin subtypes in LGSOC, HGSOC, and clear cell carcinoma cell lines. Following subcellular fractionation and whole protein isolation, western blots and RT-qPCR data were used to elucidate the molecular features of LGSOC. Western blot analysis revealed that some proteins involved in pathways linked to drug response and cellular structure, specifically EGFR, EP4, and class III beta tubulin, are upregulated in two of three LGSOC cell lines. Moreover, we found that LGSOC produces fewer and shorter McTNs relative to more aggressive HGSOC cell lines. Comparing the structural and molecular differences between LGSOC and HGSOC will identify novel targets in LGSOC and HGSOC, leading to new therapeutic approaches.

Megan Stemberger
Towson University
Mentor: Dr. Gerald Wilson

Targeted degradation of oncogenic mRNA targets using an engineered zinc finger ribonuclease

mRNAs containing AU-rich elements (AREs) often encode oncogenic factors, such as those that lead to cell proliferation and tumor growth. Tristetraprolin (TTP) is a protein that functions as a tumor suppressor by binding to and degrading mRNAs containing AREs. Suppression of TTP in many cancers contributes to disease aggressiveness. As a method of re-activating TTP function in cancer cells, expression vectors were engineered encoding the tandem zinc finger (TZF) RNA-binding domain of TTP fused to the endoribonuclease, RNase4 (R4). Control vectors encoded mutant forms of the TZF-R4 chimeric protein, including inactivating mutations in the RNA-binding domain (C147R) and the catalytic core of the nuclease (R4i). After transfecting into HeLa cells, targeted mRNA suppression by TZF-R4 was tested by qRT-PCR. To test the effects of TZF-R4 on cancer cell physiology, breast cancer cell lines that stably express the chimeric protein are being developed. Preliminary findings in MDA-MB-231 cells showed that TZF-R4 significantly attenuates cell proliferation. Cell cycle and apoptosis assays indicate that decreased cell accumulation results from delayed progression through the cell cycle rather than enhanced cell death. Future experiments will define the range of pro-tumorigenic phenotypes suppressed by TZF-R4, and identify specific mRNA targets mediating these effects.

Nina Lee
Virginia Tech
Dr. Gerald Wilson

Roles of specific domains of AUF1 in functional recognition of RNA substrates

AUF1 is an RNA-binding protein that modulates gene expression by positively or negatively regulating the stability and/or translational efficiency of its RNA targets, including many that encode oncogenic factors. However, the molecular mechanism(s) by which AUF1 mediates the fate of its RNA targets remains unknown. To begin characterizing the mechanics of RNA regulation by AUF1, this project pursued two objectives: (i) to identify AUF1 domains essential for RNA recognition and protein dimerization, and (ii) to identify RNA substrates that AUF1 robustly regulates. For the first objective, recombinant AUF1 proteins lacking N-terminal, C-terminal, and/or individual RNA Recognition Motif (RRM) domains were generated and purified. Fluorescence anisotropy-based assays confirmed that both RRMs are necessary but not sufficient for high-affinity RNA binding, while gel filtration showed that individual RRMs are not required for protein dimerization. Domain roles in stabilizing protein folding are being analyzed by chemical denaturation. From published PAR-CLIP and RNA-Seq datasets, eight candidate RNA substrates of AUF1 were selected. Clonal HEK293T cell lines expressing AUF1 shRNAs were generated to test the role of AUF1 in regulating RNA. Of six candidates reported to be stabilized or destabilized by AUF1, qRT-PCR analyses indicated an effect on expression of only one (NEAT1 lncRNA), however, AUF1 did not alter its decay rate. In contrast, two well-established AUF1 substrates, VEGF and COX-2 mRNAs, were both stabilized in shAUF1-cells. Two additional substrate candidates were reported to be translationally inhibited by AUF1. 3'-UTR-luciferase reporter assays are underway to test this hypothesis. To date, functional validation of potential AUF1 substrates identified by PAR-CLIP has yielded little confidence in the accuracy and/or interpretation of these data. This suggests that independent unbiased screens for functional AUF1 ligands may be required to identify the robust functional RNA targets essential for determining roles of AUF1 domains and interacting factors in post-transcriptional gene regulation.

Kaitlyn Zwerling
Loyola University Maryland
Mentor: Dr. Matthew Frieman

The Identification of Human Proteins, Regulating Influenza Virus Replication

Influenza virus infection is a major and recurring public health concern. Since 2010, the CDC has estimated that in the U.S, influenza infects between 10-50 million individuals annually, and results in approximately 20-50,000 deaths. While a seasonal influenza vaccine is available and moderately effective, there is a need for novel therapeutics for treating those infected. Understanding the mechanisms of the influenza viral replication is useful to identify virus-host interactions that can become therapeutic targets for influenza treatments. Using a screening platform, we have identified two host proteins, SLC16A1 and VPS29, that may affect influenza virus replication. SLC16A1, also called monocarboxylate transporter 1, functions to transport pyruvate and lactate across. It is linked to mitochondrial function, a key location for the anti-viral interferon response. Second, VPS29, a retromer involved in ER to Golgi transport, may affect viral protein transport, growth, or viral assembly. The purpose of this study is to determine how these two human proteins affect influenza virus replication. To identify where in the life cycle of viral infection these proteins function, siRNA knockdown experiments in the A549 cell line were utilized to analyze whether viral protein, RNA, or virus titer is affected. In addition, we are investigating whether SLC16A1 and VPS29 bind directly to influenza virus NS1, the protein used in the initial screen. We cloned each protein into expression vectors to conduct co-immunoprecipitation experiments to determine if influenza NS1 binds directly to each protein. We have found that reduction of SLC16A1 and VPS29 miRNA levels by siRNA transfection leads to a reduction in influenza virus production. Together, these results will provide a better understanding of the role of SLC16A1 and VPS29 in influenza viral replication, and their potential to become therapeutic targets.

Heather Buck
Towson University
Mentor: Dr. Paul Shapiro

Restoring Treatment Sensitivity to Resistant Melanoma Cells in Spheroid Culture

For individuals diagnosed with melanoma, approximately 50% possess a BRaf kinase mutation that increases the rate of cell proliferation. Persons with stage IV melanoma have a 5-year survival rate of 15-20%, which is decreased by the presence of a mutation in this enzyme. Treatments seeking to inhibit BRaf at the ATP binding site often fail due to development of drug resistance, leading to poor survival rate. The purpose of this study is to ascertain whether novel, small molecules block substrate binding sites of a down-stream extracellular signal-regulated kinase (ERK1/2) and can restore sensitivity to ATP-inhibiting treatment in melanoma cells that are resistant to the current clinically relevant combination therapies targeting mutated BRaf and MEK1/2. A375 melanoma cells containing mutated BRaf were generated to develop acquired resistance to a combination BRaf and MEK1/2 inhibitor therapy (AZD6244/PLX4032). Parent and drug resistant cells were treated with various inhibitors in doses ranging from 0.2-25uM in both monolayer and spheroid cell cultures. Monolayer cell cultures were grown via standard practice in 96-well plates. Spheroid cultures were grown in 96-well, round-bottomed, low-attachment plates. Cell viability was determined using Cell Titer Blue assay in monolayer cultures, and via Cell Titer GLO assay in spheroid cultures. Initial results indicate that cells had acquired resistance to AZD6244/PLX4032 but were still partially sensitive to ATP competitive ERK1/2 inhibitors, and that three of the tested compounds decreased cell viability in drug resistant cells but not in sensitive A375 cells in spheroid culture. Ongoing data collection is aimed at determining effects of the kinase inhibitors on viability of cells grown in monolayer versus spheroid cultures. Further evaluation of dose response on resistant cells treated with combination therapy and small molecule ERK1/2 inhibitors may indicate that targeted inhibition downstream of BRaf and MEK1/2 effectors could potentially restore sensitivity to current kinase inhibitor treatment.

Alvin Ya
University of Maryland, College Park
Dr. Sui-Seng Tee

Characterizing the Changes in Metabolic Gene Expression in Cancers with PI3K Pathway Mutations

Cancer is a disease characterized by genomic instability and mutations in cellular growth mechanisms. Mutations in the PI3K/AKT/mTOR (PAM) signaling pathway, responsible for the regulation of the cell cycle and cellular metabolism, occur frequently and are present in up to 40% of cancer cases. Recent studies have demonstrated that alterations in the PI3K pathway correlate with a worse prognosis in breast and prostate cancer. Consequently, the FDA recently approved the first PI3K inhibitor for breast cancer, alpelisib, in May 2019. In this study, we investigate the expression of cellular metabolic genes in cancer cells with mutations in the PAM signaling pathway in order to identify their potential role as a biomarker for future prognosis and treatment. Through an analysis of The Cancer Genome Atlas (TCGA) RNA-seq data we observed an upregulation in the expression of GLUT1, LDHA, and MCT4 in breast cancer samples with known amplifications in PIK3CA and deletions in PTEN. Western blotting revealed that breast cancer cell lines with mutations in PIK3CA had increased expression of MCT1 as opposed to wild-type cell lines that exhibited higher levels of MCT4. In addition, cell lines developed to be resistant to alpelisib and AZD8055, an mTOR inhibitor, were found to have decreased expression of LDHA, MCT1, and MCT4 regardless of PIK3CA status. Immunohistochemistry (IHC) was also conducted on the LuCaP series of prostate cancer patient-derived xenografts (PDXs). Tumors with known PTEN deletions exhibited a higher expression of MCT1 versus wild-type PTEN. These results demonstrate significant alterations in the expression of metabolic proteins in cancers with mutations in the PAM signaling pathway. Our observations lead to the possibility of measuring tumor metabolism using non-invasive imaging methods to stratify patients for treatment as well as to evaluate efficacy of therapy.

Sherry Fan
University of Maryland, College Park
Mentor: Dr. David Weber, Katherine Coburn

An NMR based fragment screen of heterogeneous ribonucleoprotein A18

A18 is an RNA binding protein that enhances expression of regulatory proteins necessary for the cytotoxic stress response (hypoxia, UV, cold). A18 is upregulated and promotes survival in solid tumor cells of melanoma, prostate, breast, and colon cancers, but has low expression in normal cells. This differential expression makes A18 a viable cancer drug target. Previous computational investigations of A18 in the Weber, Carrier, and MacKerell labs analyzing the X-Ray crystal structure of A18's N-terminus RNA Recognition Motif (A18N) resulted in the identification of lead compounds for small molecule inhibitors that disrupt the interaction of A18N with target mRNAs. However, due to the similarity of A18 to other RNA binding proteins, more specific small molecules need to be identified. Toward this effort, the Maybridge Ro3 small molecule fragment library was screened. To test these drug fragments experimentally, ^{15}N labeled A18N was prepared for NMR studies and the effect of the small molecules on the ^{15}N-^{1}H-HSQC NMR experiment was monitored. Each sample contained 10 mM small molecules, DMSO (5%), 0.2 mM A18N, 0.1 mM Tris buffer at pH 7.4, and D_2O (10%). HSQC NMR spectra (950 MHZ) were obtained and chemical shift perturbations (CSPs) resulting from the addition of the small molecules were determined by comparison to a control HSQC spectrum with only purified A18N. Future experiments will be done to elucidate the specific fragments that show the largest CSPs and to characterize their thermodynamic binding constant to A18N directly. The overarching goal of this project is the construction of a super inhibitor, consisting of several uniquely binding fragments linked by flexible protein linkers, that has strong and specific binding to A18N, to aid in cancer treatment.

Thomas Hill
La Salle University
Mentor: Dr. Helen Dooley

Mammalian Expression of IgNAR-Derived Binding Domains (VNARs) Targeting HER1 and HER3 Cancer Markers

Cancer cells show an exceptional ability to mutate and thereby develop resistance to drugs they encounter. As a result, there is a constant need for new therapeutics to treat drug-resistant tumors. One potential source of strong drug candidates is the shark antibody isotype IgNAR. IgNAR is a heavy chain-only antibody from which single domain molecules (VNARs) can be isolated. These VNARs are much smaller than traditional antibodies and can bind to targets in ways that are impossible for larger antibodies. Despite their small size, VNARs show high affinity and specificity for antigen binding, giving them potential application as both cancer therapeutics and diagnostics. This work focuses upon VNARs isolated from immunized nurse sharks that display high affinity for the proteins HER1 and HER3, previously verified cancer cell markers. To enable *in vitro* testing, recombinant forms of these VNARs need to be expressed in mammalian cells. To test if this is possible, I cloned the VNARs into a mammalian expression vector and transfected these constructs into 293T cells. The results of these transfections will be presented. Future work on this project will test the VNAR clones in various cancer cell viability assays and observe any inhibitory effect they have upon cancer cell growth.

Alex Greenbaum
University of Maryland College Park
Mentors: Drs. Aditi Gupta and Michal Zalzman

Regulation of chromatin state in cancer stem cells

Cancer stem cells (CSCs) are rare tumor cells that maintain the ability to self-renew, drive tumor growth, and contribute to therapeutic resistance and tumor recurrence. Here, we investigated the role of Zinc finger and SCAN domain containing 4 (ZSCAN4) in head and neck squamous cell carcinoma (HNSCC) and its potential role in CSC. The mouse *Zscan4* is an early embryonic gene involved in telomere and genomic stability of embryonic stem (ES) cells. In mouse ES cells, *Zscan4* was shown to restore cell developmental potency, and to facilitate the reactivation of early embryonic genes. Consistent with these reports, it was found to be involved in nuclear reprogramming. Additional reports suggest that *Zscan4* expression positively correlates with chromatin de-repression. Data from our lab indicate that the human ZSCAN4 is expressed in HNSCC and is enriched for in CSCs. Like other embryonic genes, ZSCAN4 has been proposed to have significance in cancer. However, its activity in CSCs remains unknown. Cancer cells are characterized by more open and permissive chromatin signatures, enriched in specific active histone marks. Data from our laboratory indicate that ZSCAN4 interacts with the histone deacetylase HDAC1. We hypothesized that ZSCAN4 exerts its effect on the chromatin by destabilization of HDAC1, sending it to degradation, and thereby, leading to histone hyper-acetylation and de-repression. Therefore, we utilized our doxycycline inducible ZSCAN4 HNSCC cell lines in which overexpression of ZSCAN4 can be triggered by addition of doxycycline to the culture medium. To induce ZSCAN4, cells were either treated or remained untreated with doxycycline for 48 hours. Next, cells were incubated with cycloheximide, harvested and assessed for the effect of ZSCAN4 on the half-life of HDAC1 by western blot. Defining the mechanism by which ZSCAN4 acts in cancer is important for the development of drugs designed to target cancer stem cells.

Lauren Sands
Wake Forest University
Mentor: Dr. Silvia Montaner

The role of ANGPTL4 (Angiopoietin-like 4) in head and neck squamous cell carcinoma (HNSCC) metastasis

Head and neck cancer is the sixth most common cancer worldwide with around 550,000 new patients diagnosed each year. The majority of head and neck cancers are squamous cell carcinomas (HNSCC). Lymph node metastasis is a key factor in determining the prognosis of individuals with HNSCC, and often the disease has spread to the sentinel lymph nodes prior to diagnosis. Once the cancer has metastasized to the lymph nodes, the five year survival rate drops from 90% for patients without metastasis to less than 40%. Interestingly, the expression of angiopoietin-like 4 (ANGPTL4) has been shown to be elevated in HNSCC tumor specimens, specifically in oral and esophageal cancers. ANGPTL4 is a known angiogenic and vessel hyperpermeability factor. Based on our preliminary data, we hypothesized that ANGPTL4 stimulates lymphangiogenesis to promote tumor cell metastasis. By modeling the process of lymphangiogenesis ex vivo, we sought to determine the effects of exogenous ANGPTL4 on primary lymphatic endothelial cells (LECs). This involved interrogating ANGPTL4-stimulated cellular responses, including migration, proliferation, and tubule formation. We also studied ANGPTL4-stimulated signaling pathways that could be involved in these mechanisms, including MAPKs, mTOR, and Akt pathways.

Sophie Nerone
Wheaton College
Mentor: Dr. Ashkan Emadi

Targeting Pancreatic Adenocarcinoma Using a Combination of Pegcrisantaspase and the Bcl-2 Inhibitor Venetoclax

Pancreatic adenocarcinoma is a lethal disease with a 5-year survival rate of approximately 10%, largely due to a lack of early detection methods and the emergence of chemo-resistance. Asparaginase, an enzyme that hydrolyzes asparagine and glutamine to aspartate and glutamate, respectively, is well-established for the treatment of acute lymphoblastic leukemia (ALL). Recently, Emadi et al. reported that asparaginase-induced glutamine depletion can be used safely and effectively for treatment of acute myeloid leukemia (AML). Similar to leukemia cells, pancreatic cancer cells are also glutamine-dependent. A recent Phase 2b clinical trial demonstrated that second-line treatment of metastatic pancreatic cancer with a combination of chemotherapy and eryaspase (asparaginase encapsulated in erythrocytes), reduced the risk of death by 40%. Inhibition of glutaminolysis or a decrease in plasma glutamine concentration have been shown to activate mitochondrial apoptotic pathways in pancreatic adenocarcinoma. Bcl-2 is a major anti-apoptotic protein and the Bcl-2 inhibitor Venetoclax is FDA-approved for AML. We hypothesize that in pancreatic adenocarcinoma, glutamine depletion induced by pegcrisantapase (PegC), a longer-acting pegylated asparaginase derived from *Erwinia chrysanthemi*, will lead to the activation of mitochondrial apoptosis pathways and synergize with Bcl-2 inhibition induced by Venetoclax. To investigate *in vitro*, we assessed cell proliferation in pancreatic cancer cell lines, MiaPaca-2 and BxPC3, in response to Venetoclax or PegC treatment to determine the IC_{50} of each drug, followed by potentiation studies. We report that while PegC significantly impacts cell proliferation in both cell lines tested, Venetoclax was not as effective in the cell line that has a K-ras mutation. Ongoing work is focused on determining whether Venetoclax could potentiate the cytotoxicity of PegC. Furthermore, we are investigating whether or not PegC, Venetoclax, and their combination have synergism with the standard of care chemotherapies, gemcitabine and erlotinib.

Christina Kratzmeier
University of Maryland, College Park
Mentor: Dr. Rena Lapidus and Kristin Maloney

Sequence Validation of the *FLT3* F691L Point Mutation in AML Cells

The FMS-related tyrosine kinase 3 gene, *FLT3*, encodes a class 3 receptor tyrosine kinase mainly found in hematopoietic cells that is activated upon binding the FLT3 ligand. Mutations causing constitutive activation of *FLT3*, namely an internal tandem duplication (ITD) and ones at codon Asp835 in the tyrosine kinase domain (TKD), have been identified in acute myeloid leukemia (AML) and are targets for therapy. A different variant in the TKD, p.Phe691Leu (F691L) was found to confer resistance to the recently FDA approved *FLT3* inhibitor, gilteritinib, in the presence of a *FLT3*-ITD. In this study, we sought to develop a Sanger sequencing assay to detect the *FLT3* F691L mutation to promote personalized therapy in AML patients. First, we validated resistance of the gilteritinib resistant cell line (MOLM14-F691L) by generating an IC_{50} with gilteritinib and comparing to parental MOLM-14 cell line. Secondly, we sequenced exon 17 of *FLT3*, (NM_004119.2) which contains codon 691. We isolated DNA from the parental and resistant lines, amplified specific region of the genome using PCR, and ran gel electrophoresis to isolate and purify the PCR product from both cell lines. Next, the purified PCR product was sequenced using Sanger sequencing in order to identify any sequence alteration in the gilteritinib resistant line. The IC_{50} of the MOLM14 parental cells was 6.25 ± 2.76 nM while the IC_{50} of the Gilterinitib resistant MOLM14 F691l was 25.88 ± 4.12 nM leading to a 4.14-fold change. Analysis of the sequencing data revealed the c.2071T>C variant that leads to an amino acid change of phenylalanine to leucine. These data demonstrate that we indeed have a gilteritinib resistant AML cell line and that the mutation in the resistant line was verified. Future work will include validating this assay in a clinical lab so that patient samples can be evaluated for this important mutation.

Nicole Illesca
Bryn Mawr College
Mentor: Dr. Feyruz Rassool

DNA Double Strand Break Repair Capacity in AML after HDAC Inhibition

In leukemias, specific oncogenic proteins engage histone deacetylase (HDAC) to their target gene's promoter. This in turn contributes to leukemogenesis which suggests that these diseases are great candidates for treatment with HDAC inhibitors. HDACis are an epigenetic modification that downregulate DNA expression by removing acetyl groups from an amino acid on a histone. It is also known that HDACis trap a methylation enzyme, DNMT1, into the DNA, forming a DNMT-DNA complex that is resolved by Poly ADP-Ribose Polymerase 1 (PARP1), a DNA repair protein. Inhibition of PARP1 blocks the resolution of the complex which previous research has found to lead to the formation of DNA double strand breaks (DSBs). DSBs are extremely lethal to cells and lead to apoptosis. To further explain the mechanism by which HDACis function, we are interested in determining if HDAC inhibitors may also directly inhibit DNA DSB repair, and therefore lead to cell death, by altering expression of repair genes through their impact on methylation and acetylation. To achieve this, three assays were performed that test for DSB repair efficiency: homologous recombination (DSB repair during replication), non-homologous end joining (DSB repair outside of replication), and alternative NHEJ (a minor PARP-dependent pathway). If HDAC inhibitors reduce DSB repair capacity in AML, they may also sensitize cells to PARP1 inhibition and lead to apoptosis through a concept known as synthetic lethality. Future studies looking at combination therapy of HDACi and PARPi will yield insights into the mechanisms through which PARPi sensitivity and therefore synthetic lethality occurs.

Emilee Stenson
State University of New York at Fredonia
Mentor: Dr. A-Lien Lu-Chang

The role of DNA binding activity of cell cycle checkpoint protein Rad9 in DNA repair

Defects in DNA repair and DNA damage response (DDR) can lead to genomic instability which contributes to premature aging and carcinogenesis. DDR provides surveillance mechanisms and controls cell survival. It has been suggested that the checkpoint clamp Rad9-Rad1-Hus1 (9-1-1) simultaneously activates the DDR and coordinates DNA repair pathways to ensure accurate and timely DNA repair following DNA damage. 9-1-1 is essential for embryonic development and its expression is associated with cancer development and treatment. We have shown that each subunit of 9-1-1 plays a distinct role in coordinating DNA repair and DDR. We hypothesize that DNA binding by Rad9 is essential for DNA repair. The goal of this project is to determine how mutations in the inner ring residues of Rad9 protein impact DNA binding and affect cell's sensitivity to DNA damaging agents. Wild type (WT), R150A/R223A mutated (DM), and amino acids Δ160-163 (LD) deleted Rad9 (residues 94-266) proteins purified from *E. coli* were analyzed for DNA binding affinity by performing a gel mobility shift assay. $Rad9^{DM}$ protein has decreased DNA binding and $Rad9^{LD}$ protein has no DNA binding as compared to $Rad9^{WT}$. Human PC3 and *Rad9* knockout (KO) PC3 cells were transfected with either a control vector or a vector containing WT, DM or LD intact Rad9. Cells were treated with increasing concentrations of H_2O_2 and examined for cell viability. *Rad9* KO cells are more sensitive to H_2O_2 than control PC3 cells. *Rad9* KO cells expressing $Rad9^{DM}$ protein are more sensitive to H_2O_2 than cells expressing $Rad9^{WT}$ protein. Our results indicate that DNA binding by the Rad9 protein in the 9-1-1 clamp is critical for genome protection against reactive oxygen species. Because tumor cells rely on DNA repair and DDR for proliferation and survival, these pathways are attractive targets for novel anticancer drugs.

Blair Landon
University of Maryland, Baltimore County
Mentors: Drs. Winkles, Kim, and Woodworth

Development of a blood brain barrier model for screening brain tumor nanotherapeutics

Glioblastoma (GBM) is an aggressive type of adult brain cancer with a current life expectancy of 12-18 months after diagnosis. One of the greatest challenges in the treatment of this disease is that very few cancer drugs can pass through a structure in the brain called the Blood Brain Barrier (BBB). The BBB is formed early in development and consists of endothelial cells connected by tight junctions. Astrocytes and pericytes also contribute to the structural integrity of the BBB. It has been reported that >90% of all small molecule cancer drugs and nearly 100% of larger protein-based therapeutics (e.g., antibodies) do not cross this barrier. Our laboratory is developing a nanoparticle-based drug delivery platform for treatment of primary and metastatic brain tumors. The aim of this project was to establish an *in-vitro* model that mimics the BBB and can be used to assess the transport of different types of drug-loaded nanoparticles across the BBB. Our model consists of mouse brain endothelial cells (bEND.3) and mouse astrocytes (C8D1A) plated on different sides of a 0.4 µm pore size membrane located within a transwell culture dish. Barrier strength was assessed by Trans Epithelial Electrical Resistance (TEER) measurements using EVOM2 Epithelial Voltmeter and STX2 chopstick electrodes. TEER measurements stabilized two days after co-culture seeding. Immunofluorescence labelling for occludin confirmed the presence of tight junctions on bEND.3 cells. Barrier function was quantified through fluorescence tracer assays using a 3 kDa FITC conjugated dextran. The presence of a co-culture reduced the transit of dextrans across the transwells when compared to wells containing no cells. Currently, experiments are in progress to assess nanoparticle transport across our BBB model.

Darien Campisi
University of North Carolina at Chapel Hill
Mentors: Drs. Atanu Chakraborty and Ryan M. Pearson

Development of a safe and effective non-viral gene delivery platform targeted to immune cells

The delivery of nucleic acids to immune cells has widespread potential to correct the genetic abnormalities present in numerous diseases. Current methods, relying on physical or viral approaches, are limited in potential for clinical application by high toxicity, inability to be modified for cell-specific targeting, and unwanted genetic mutations and immunogenicity. Non-viral gene delivery has the potential to overcome many limitations, namely the issues with toxicity and cell-specific targeting. Here, we investigated how the physicochemical properties of a non-viral gene delivery system affect the transfection efficiency of immune cells. This delivery system is comprised of a hyperbranched positively-charged polyethylenimine (PEI) core and plasmid DNA (green fluorescent protein; GFP), which form polyplexes through electrostatic interaction. Using layer-by-layer assembly, the polyplex is coated with negatively-charged poly(ethylene-alt-maleic acid) (PEMA) to reduce toxicity and non-specific interactions. Experiments were done to decrease polyplex size to <300 nm by varying the percentage of PEMA, the ratio of PEI to plasmid DNA, and other formulation conditions. Polyplex transfection efficiency was determined *in vitro* using bone marrow-derived macrophages and Jurkat cells. Transfection efficiency (GFP expression) was measured using fluorescence microscopy and flow cytometry. The stability of polyplexes in the absence and presence of serum proteins was evaluated using DNA gel electrophoresis. The transfection efficiency was dependent on the N/P ratio and level of PEMA coating. Transfection levels were highest in polyplexes with an N/P ratio of 40 and 10 wt% PEMA. Incubation of the polyplexes with high levels of serum did not result in plasmid migration, showing potential for *in vivo* use. In conclusion, PEI-DNA polyplexes coated with PEMA are able to transfect immune cells efficiently. Through *in vivo* studies and the addition of immune cell-targeting ligands, this gene delivery system can be developed for efficient and specific delivery of nucleic acids to immune cells.

Richa Beher
University of Maryland College Park
Mentor: Dr. Gregory Szeto

Inflammasome Activation Induces Spatial Regulation of Metabolic Enzymes in Tumor Cells

Glucose plays important roles in both energy metabolism and building block biosynthesis, the former which requires oxygen and the latter which occurs when oxygen supply is limited. In proliferating cancer cells, glucose metabolism is dysregulated in that building block biosynthesis is initiated even in the presence of oxygen. Previous studies have shown that cancer cells undergoing metabolic stress rapidly form cytoplasmic clusters of glycolytic enzymes hypothesized to increase the cells' efficiency in utilizing scarce resources. We hypothesize that extracellular ATP contributes metabolic stress through induction of inflammation to cause glycolytic enzyme clustering via activation of the NLRP3 inflammasome. HeLa cells stably transfected with human liver-type phosphofructokinase (PFKL)-eGFP, a rate-limiting enzyme in glycolysis, were incubated alone and concurrently with ATP, 2-deoxyglucose (2DG), and Glybenclamide, an inhibitor of the NLRP3 inflammasome. Confocal microscopy was used to identify PFKL clustering events defined by an area greater than 0.1 um^2 and less than 8 um^2. 1-hour incubation with 5 mM of ATP and 25 ug/mL of Glybenclamide resulted in fewer clustering events and smaller cluster size compared to induction by ATP alone. HeLa cells incubated for 6 hours with 25 mM 2DG and Glybenclamide showed no significant change in the number or size of clustering events compared to cells incubated with 2DG alone. An ELISA quantification of secreted IL-1β in the supernatants of the treated HeLa cells will be used to confirm activation of the NLRP3 inflammasome. Results from these experiments connect cancer cell inflammation and metabolism, providing a novel mechanism for inflammation to enhance cancer cell growth through metabolic adaptation. Ongoing studies xenografted tumors into mice to determine if, where, and to what degree clustering occurs within growing tumors. Understanding the mechanism for and the effects of glycolytic clustering on cancer cell interactions with other cells, specifically immune cells, in the tumor microenvironment are crucial to developing therapies that can either promote or impair tumor growth depending on the context.

Laura Gerrick
Stevenson University
Mentor: Dr. Alexander Drohat

The Role of N191 in acid-catalyzed extraction of 5-carboxylcytosine by Thymine DNA Glycosylase

Thymine DNA glycosylase (TDG) is a base excision repair (BER) enzyme responsible for initiating the repair of G:U and G:T mismatches that result from deamination of cytosine and 5-methylcytosine (5-mC) respectively. TDG also plays an important role in regulating gene expression by aiding in the removal of epigenetic DNA methylation patterns. 5-mC is an epigenetic mark that is associated with gene silencing. Erasure of 5-mC begins with TET enzymes, which oxidize 5-mC to 5-hydroxymethylcytosine (5-hmC), and then to 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC). TDG excises these modified bases to leave an abasic site, which is converted to cytosine by follow-on BER enzymes, thus completing active DNA demethylation. Due to the differences in leaving group stability for 5-fC and 5-caC they are excised via different mechanisms, with the latter requiring protonation in order to extract the base. For 5-caC this protonation is hypothesized to be facilitated by asparagine191 of TDG. This study aimed to assess the activity of point mutations at N191 for TDG82-308 to explore its role in the acid-catalyzed excision of 5-caC. Alanine and lysine mutants were overexpressed, purified through nickel affinity, ion exchange, and size exclusion chromatography, and the mutational effect on glycosylase activity was determined through single turnover kinetics using G:T, G:fC, and G:caC DNA substrates. The results are expected to inform the site of protonation on 5-caC prior to its excision. Future studies will aim to create new mutations at this side chain as well as obtain crystal structures of the mutant enzyme in complex with G:caC DNA to further investigate the mechanism for excision of 5-caC.

Mariah Lee
Newberry College
Mentor: Dr. Achsah Keegan, Dr. Svetlana Chapoval

Human Treg cells express Plexin B1 and Neuropilin 2, two functional receptors for neuroimmune semaphorins

Semaphorin 4A (Sema 4A) is known to be a neuronal guidance protein, but recent research has led to understanding Sema 4A's function in the immune system. Sema 4A has also been shown to aid in T cell activation as a "third" co-stimulatory molecule. It is both a soluble and transmembrane protein that forms essential homodimers before binding to various co-receptors, such as neuropilins, Tim-2, ILT-4, and Plexin –D1, -B1, -B2, and –B3. A recent report from our laboratory suggested that Sema4A stabilizes human Treg cell phenotype through the co-receptor Plexin B1. However, other reports suggest that Neuropilin 1 expressed on Tregs is critical for their stabilization in mice bearing tumors. Therefore, in this study we examined the expression pattern of Sema4A receptors by flow cytometry. We performed staining of human peripheral blood mononuclear cells (PBMC) with a set of markers for Treg cell identification (CD3, CD4, CD25, Foxp3, Helios, and CD127) and analyzed the expression of Plexins B1 and B2 and Neuropilins 1 and 2. We confirmed the expression of Plexin B1 on Treg cells as well as the lack of Neuropilin 1. In addition, we found that human Treg cells in the steady state condition express Neuropilin 2 but not Plexin B2 or Plexin B3. This study eliminates Plexin B2 and Neuropilin 1 as potential players in Sema4A-induced Treg cell stabilization. Moreover, it paves the way for further analysis of Neuropilin 2 function on human Treg cells and its potential interaction with Sema4A.

Ongoing experiments using the colorectal carcinoma cell line HCT116 are designed to identify the Sema4A counter receptor responsible for mediating signal transduction. Our goal is to examine changes in protein phosphorylation and expression after Sema4A treatment by Western blot analysis. If Plexin B1 is the active signaling receptor, we expect to see a loss of Akt phosphorylation after treatment with soluble Sema4A that is prevented by anti Plexin B1 antibodies.

Studying specific subset of immune cells and their signaling receptors can aid in further understanding of this neuro-immune protein and its function within the immune system. Explicitly, the Sema4A-Treg interaction may pose new therapeutic targets in cancer progression, angiogenesis, asthma, and autoimmune disease.

Sara Fioretti
University of Mary Washington
Mentor: Dr. Nevil Singh

Brewing up a Storm: Modeling Anti-CD-19 CAR T-cell Adverse Side Effects in Mice

Immunotherapy using Chimeric-Antigen-receptor (CAR) T-cells is a promising approach to treat cancers. In the case of B cell lymphomas which express the surface marker CD19, CAR T-cells (against-CD19, marketed as YESCARTA), are shown to produce high rates of sustained complete responses. However, success is limited by severe autoimmune adverse side effects. 35-96% of patients experience mild to severe cytokine release syndrome (CRS) with 60% of those patients experiencing some form of neurotoxicity. In addition, this therapy also eliminates healthy B cells which express CD19. The etiology of these adverse events are not well understood. For instance, we know that CAR related CRS is correlated with increased IL-1β, IL-6, IL-12, and TNF-α secreted by innate cells, and GM-CSF and INF-γ secreted by T-cells. But causal relationships between these inflammatory innate cells and the CAR T-cells are not well-defined. Towards approaching these questions using genetic tools, robust mouse models which not only show the anti-tumor effect of CAR T cells but also enable us to study the full spectrum of adverse effects are required. I will discuss my efforts to develop one such model, over this summer. Based on published literature, our central hypothesis was that the development of adverse events in a mouse model for CD19-CAR T cell therapy depends on the level of CD19 expression in healthy B cells. My approach involved (1) generating CD19-CAR T cells by retroviral transduction of primary mouse T cells (2) evaluation of their ability to respond to healthy B cells, (3) characterization of cytokines elicited directly by the CAR-T cells and (4) evaluating the effect of CD19 dose in determining the extent of 2 & 3. I will also discuss how the conditioning regimen used typically used for CAR-T transfers may also contribute to the severity of the adverse side effects.

Ben Atkinson
Loyola University Maryland
Mentors: Dr. Xuefang Cao and Ellis Tibbs

Analysis of Granzyme B Expression in a Murine Ex Vivo Model of Bone Marrow Derived MDSC's

Suppression of the immune system, by tumors, contributes to cancer progression, and poses a serious roadblock for cancer immunotherapies. A major method for suppressing the immune system involves the development and recruitment of a highly suppressive class of cells, called myeloid derived suppressive cells (MDSCs). MDSCs encompass a diverse population of immature myeloid cells that have been aberrantly stimulated by tumors, and utilize a vast repertoire of mechanisms to suppress the immune response in the tumor microenvironment (TME). Many of the suppressive mechanisms utilized by MDSCs have been identified and studied, but little research has been conducted to identify granzyme B (GzmB) production in MDSCs. It is known that GzmB, when produced by other suppressive cells, such as B regulatory cells, contributes to the inhibition of tumor infiltrating T lymphocytes' (TILs) activation, and promotes tumor progression. Targeting the down regulation of MDSCs and their suppressive mechanisms is a potential therapeutic approach that could enhance the effect of TILs.

We hypothesized that MDSCs do produce GzmB. To test this hypothesis, we used ELISAs and RT-qPCR to measure GzmB expression in murine ex-vivo and in-vitro MDSCs cultured in different cytokine stimulations and cancer cell line media. We compared the GzmB expression of MDSCs derived from wild type (WT) mice to MDSCs from GzmB knockout mice. ELISA analysis found significantly higher expression of GzmB in the WT MDSCs compared to the knockout group.

These results add data to the growing argument that MDSCs do produce GzmB. Future studies can confirm these findings through more precise technologies, such as flow cytometry. In addition, further studies should focus on how GzmB promotes tumor progression and suppresses TILs.

Perry Summers
Bucknell University
Mentor: Dr. Tonya Webb

Elucidating the role of Bcl-xL on CD1d-mediated NKT cell activation

Natural killer T (NKT) cells are a subset of T cells that play an important role in host anti-tumor immune responses. NKT cells are activated by glycolipids presented by CD1d molecules on antigen presenting cells (APCs). Upon activation, NKT cells can directly kill tumor cells and produce a wide variety of cytokines to further enhance anti-tumor immunity. However, the mechanisms by which NKT cells recognize malignant cells remain unclear. It is known that cancerous cells upregulate pro-survival factors such as B-cell lymphoma-extra large (Bcl-xL), which inhibits programmed cell death and aids in cancer cell proliferation. Previous studies in the lab have shown that overexpression or induction of Bcl-xL in APCs increases their ability to activate NKT cells. In this study, we sought to determine the mechanism by which Bcl-xL overexpression enhances CD1d-mediated NKT cell responses. LMTK and WEHI-231, murine fibroblast and B cell lymphoma cell lines, respectively, were used to establish stable Bcl-xL overexpressing cell lines. To assess differential protein expression in Bcl-xL overexpressing cell lines, compared to controls, we used label-free quantitative mass spectrometry. 36 proteins were similarly upregulated or downregulated in both WEHI-231 and LMTK cell lines and eight candidate proteins were selected for validation: Brd7, Ppp3cb, Pbx1, Iqsec1, Snx7, Spcs1, Wdr81, and Mgll. Quantitative PCR and western blot analysis were used to validate these targets. Following confirmation of our targets, we will alter their expression in LMTK and WEHI cell lines using genetic manipulation or pharmacological inhibition and determine the impact of these factors on CD1d-mediated NKT cell activation. Collectively, these studies will aid in the identification of proteins critical for NKT cell recognition of cancerous cells, which may lead to the development of novel immunotherapeutic targets.