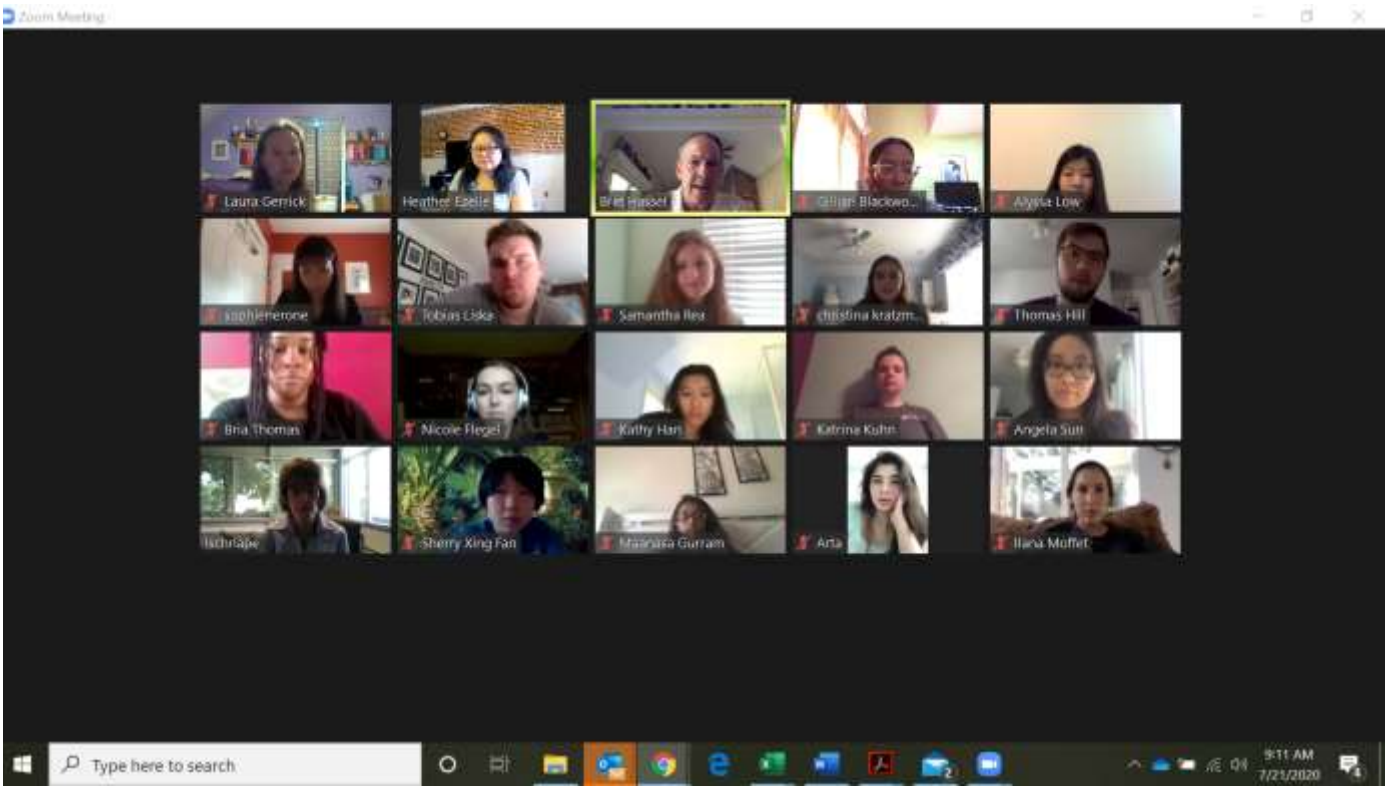
The OSR Student Research Forum: 2020 Nathan Schnaper Intern Program in Translational Cancer Research



July 29 & 31, 2020
Presented virtually on Zoom

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

NCI Cancer Center
A Cancer Center Designated by the National Cancer Institute

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The Marlene and Stewart Greenebaum Comprehensive Cancer Center,
Dr. Kevin Cullen, Director
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Bench-to-Bedside Lecture Series speakers
NSIP alumni and MSTP student panelists and presenters
GPILS student cancer research presenters
-AND-
The 2020 NSIP mentors!

NSIP
Nathan Schnaper Intern Program
in Translational Cancer Research

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

OSR Student Research Forum:
2020 NSIP Research Symposium Session 1

Wednesday, July 29, 2020
10:30 am to 12:00 pm
https://umaryland.zoom.us/j/95536648006?pwd=L2xJNlVxYUVQaXNiWVRuUEZtOG16UT09
Passcode: Schnaper

	Speaker	Mentor
10:30 am	**Bret A. Hassel, PhD** *Director's Welcome*	
10:30 to 10:40 am	**Maanasa Gurram** *Discovery Orientation and Science Attitudes Among Middle School UMB CURE Scholars*	Elizabeth Parker
10:40 to 10:50 am	**Katherine Han** *Impact of Gamma-Chain Signaling on CD4 Memory T-Cells*	Nevil Singh
10:50 to 11:00 am	**Alyssa Low** *Investigating Gene Signatures of T Cells Stimulated Under Chronic and Acute Conditions*	Nevil Singh
11:00 to 11:10 am	**Tobias Liska** *Herpes Simplex Virus Type-2 shedding and genital ulcers during early HIV in Zimbabwean women*	Rebecca Nowak
11:10 to 11:20 am	**Laura Gerrick** *Elucidating the role of CD1d-mediated antigen presentation on immune responses to cancer immunotherapy*	Tonya Webb
11:20 to 11:30 am	**Gillian Blackwood** *The Impact of Ethnicity on Single Nucleotide Polymorphisms (SNPs) that Regulate Immune Signatures in Prostate Cancer*	Tonya Webb
11:30 to 11:40 am	**Christina Kratzmeier** *Impact of Ethnicity-Related SNPs in the Regulation of AMPK-mTOR Signaling in Prostate Cancer*	Tonya Webb
11:40 to 11:50 am	**Beza Ketema** *Racial Disparities in Breast Cancer Outcomes*	Paula Rosenblatt
11:50 am to 12 pm	**Nicole Flegel** *Impact of Radical Surgery vs. Radiotherapy on Prostate Cancer Specific Mortality and Overall Mortality in Men with Ductal Prostate Cancer*	Minhaj Siddiqui

OSR Student Research Forum:
2020 NSIP Research Symposium Session 2

Friday, July 31, 2020
10:30 am to 12:00 pm
https://umaryland.zoom.us/j/95679567467?pwd=bGRWbkxBSjRrN0Z6VkJLWmFNOGpDUT09
Passcode: Schnaper

	Speaker	Mentor
10:30 am	**Bret A. Hassel, PhD** *Director's Welcome*	
10:30 to 10:40 am	**Sherry Fan** *Identification of Novel Genetic Susceptibility Loci for Thoracic and Abdominal Aortic Aneurysms Via Genome-Wide Association Studies of the UK Biobank*	Charles Hong
10:40 to 10:50 am	**Sophie Nerone** *Identifying transcription factor markers of nuclear heterogeneity in glioblastoma*	Heather Ames
10:50 to 11:00 am	**Katrina Kuhn** *ChIP-Sequencing Analysis of ZSCAN4 Reveals its Chromatin and Gene Targets*	Michal Zalzman
11:00 to 11:10 am	**Ilana Moffet** *Antioxidants as an Adjuvant Therapy in Cancer Patients undergoing Chemotherapy with a Curative Intent*	Cheryl Knott
11:10 to 11:20 am	**Arta Kasaeian** *Targeting Serine Metabolism in combination with Glutamine Depletion for Acute Myeloid Leukemia*	Ashkan Emadi
11:20 to 11:30 am	**Thomas Hill** *Identifying Complement Proteins in Sharks: The Oldest Ancestor of the Modern Immune System*	Helen Dooley
11:30 to 11:40 am	**Samantha Rea** *Protein Expression Patterns in Monolayer and Spheroid Melanoma Cell cultures with Acquired Resistance to BRaf and MEK1/2 Inhibitors*	Paul Shapiro
11:40 to 11:50 am	**Bria Thomas** *Measuring Financial Toxicity of Cancer in Young Active-Duty Military Patients*	Christabel Cheung

11:50 am to 12 pm	**Angela Sun** *Probing the SIX proximal interactome for functional protein interactions*	Tami Kingsbury

Abstracts

(in speaking order)

Maanasa Gurram
University of Maryland College Park
Mentor: Dr. Elizabeth Parker

Discovery Orientation and Science Attitudes Among Middle School UMB CURE Scholars

Research has shown that the burden of cancer is disproportionate within minority communities. A possible solution that has shown promise in increasing minority representation in healthcare fields is pipeline programs, which are designed to support and guide a specific subset of individuals to increase retention in biomedical career paths. The NCI's Center to Reduce Cancer Health Disparities established the Continuing Umbrella of Research Experiences (CURE) program to serve as a pipeline for underrepresented populations to careers in biomedical research and healthcare. The CURE program has been implemented at the University of Maryland Baltimore (UMB). Previous studies on similar pipeline programs explored the discovery orientation of the participants. Discovery orientation refers to a measure of affect that indicates level of curiosity, and is used as an indicator of interest and aptitude for science-related subjects. This study explored how discovery orientation and science interests differ between males and females participating in the UMB CURE Scholars program. The previous study found that discovery orientation scores were higher for males, which led to the hypothesis that the male students in the UMB CURE program would exhibit higher discovery orientation. The sample of middle school students (grades 6^{th}-8^{th}) completed a cross-sectional survey containing questions measuring discovery orientation. The sample was 100% black and the majority of participants were males (54%) in 7th grade (42%). Though not statistically significant, discovery orientation subscores were either equal or higher for females than males, refuting the hypothesis. In addition, the overall mean scores for subscales were higher in our sample compared to the published results. Limitations of this study include a small sample size and the cross-sectional analysis limits causality. Future studies should explore possible explanations to the higher discovery orientation exhibited by females and examine how participation in STEM related programs improves overall science identity.

Kathy Han
Wellesley College
Mentor: Dr. Nevil Singh

Impact of Gamma-Chain Signaling on CD4 Memory T-Cells

T-cells are important players of the adaptive immune system and have two main types: cytotoxic T-cells (expressing the surface marker CD8) and helper T-cells (expressing CD4). Both are important for recognizing and riding the body of foreign or abnormal entities and can be maintained as memory T-cells to set a higher baseline for future immune responses. Understanding the mechanisms to maintain memory T-cells allows us to improve vaccines, immunotherapies, and other treatments dependent on leveraging T-cell memory. Based on studies mostly using CD8 T-cells, memory T-cells appear to utilize cytokines IL-2, IL-7, and IL-15 to stay alive. These cytokines, also important for T-cell development, have multi-part receptors: one of the parts, the common-gamma-chain, is crucial to successful signaling. Previous research found that in gamma-chain knockout (GC-KO) mice, CD8 memory T-cells were absent, while CD4 memory T-cells were present. This was unexpected and suggests that our current model of IL-2, IL-7, and IL-15 cytokines controlling memory is perhaps limited to CD8 cells and that there is a separate model for CD4 memory T-cells. To investigate whether memory CD4s or CD8s in the GC-KO mice were similar to memory T-cells from normal mice, we FACS-sorted these T-cells and obtained RNA-seq data from GC-KO CD4 memory T-cells and wild-type CD4 memory T-cells. Using R-Studio, I conducted differential gene expression analysis between the two to identify the most differentially expressed genes which I then used to identify enriched pathways of GC-KO and wild-type memory T-cells via GSEA. I found downregulation of the FOXP3 gene and TGF-beta cytokine family and decreased activation of the PI3K signaling pathway in GC-KO cells, indicating some differential development between GC-KO and wild-type CD4 memory T-cells. Further research can be done to confirm CD4 memory T-cells GC independence and identify a new cytokine model for CD4 memory T-cells.

Alyssa Low
Texas A&M University
Mentor: Dr. Nevil Singh

Investigating Gene Signatures of T Cells Stimulated Under Chronic and Acute Conditions

T cells are a crucial component of the adaptive immune response and play fundamental roles in activating and directing the response of other immune cells following pathogen recognition. In an acute pathogen encounter, these T cells are stimulated and perform their effector functions to clear the pathogen within weeks. However, in cases of prolonged antigen stimulation with chronic infections and cancer, this capability of T cells is reduced, resulting in exhausted T cells with decreased antigen control. While this phenomenon may have a role in reducing subsequent immunopathology and limiting autoimmunity, the consequences of failing to achieve clearance can be detrimental. We hypothesized that genes that are differentially expressed between chronically and acutely stimulated T cells will help identify molecular pathways responsible for reducing T cell function in the former. To evaluate this hypothesis, we examined gene expression differences between these T cells using a mouse model. The approach involved transferring naïve T cells with a homogenous TCR into two populations of mice. One set of mice had the cognate antigen permanently expressed, modeling a chronic stimulation; in the other set, the T cells were stimulated by an immunization, modeling an acute infection. After 28 days, these T cells were purified by FACS sorting, RNA isolated, and subjected to microarray analysis. GenePattern, R Studio, and Bioconductor modules were used to analyze these data. Differential gene expression analysis was performed and subsequently evaluated by Gene Set Enrichment Analysis. Signatures of T cells in chronic versus acute stimulated environments were developed and compared against published datasets to analyze common activity. Such pathways can serve as drug targets to improve immunity against tumors. Broadly, this understanding of gene expression changes in chronic and acute T cells can grant continuing insight into how an exhausted phenotype may be overcome and inform therapeutic measures.

Tobias A. Liska
Assumption University
Mentor: Dr. Rebecca G. Nowak

Herpes Simplex Virus Type-2 shedding and genital ulcers during early HIV in Zimbabwean women

Herpes Simplex Virus Type-2 (HSV-2) through inflammation and recruitment of immune cells is speculated to increase risk of incident HIV 3-5 fold. However, prior studies used seropositivity, an antibody marker of lifelong infection, as opposed to indicators of active replication of HSV-2. Our objective was to assess whether HSV-2 viral shedding or genital ulcers preceded HIV acquisition. The Hormonal Contraception HIV cohort recruited women from the general population in Zimbabwe, Uganda and Thailand. Women who acquired HIV (n=134) were matched to HIV-negative women (n=266) from Zimbabwe on follow-up time, age, and sexually transmitted infections. All women had either incident or prevalent HSV-2 by serology. Up to 5 quarterly visits bracketing the index visit of HIV acquisition ($t_{-6months}$, $t_{-3months}$, t_{index}, $t_{+3months}$, $t_{+6months}$) were selected for real-time PCR detection of HSV-2 viral DNA. Any self-reported and clinician diagnosed ulcers were binary categorized. Binomial models were used to estimate prevalence ratios with 95% confidence intervals at each visit interval. Proportional differences by eventual HIV-seroconversion status were evaluated using Chi-square and Fisher's exact tests. Detection of any viral shedding irrespective of time was higher among seroconverters as compared to HIV-negative women (26% vs. 14%, $p<0.01$). Prevalence of viral shedding began to increase for the seroconverters as compared to the HIV-negative women at $t_{-3months}$ (5.9% vs. 2.1%, $p=0.06$), peaked at seroconversion (9.9% vs. 4.0%, $p=0.02$), and no longer differed at $t_{+3months}$ (9.9% vs. 5.5%, $p=0.14$). Genital ulcers peaked in prevalence at $t_{+6months}$ for the seroconverters as compared to HIV-negative (11% vs. 0.5%, $p=0.001$). Overall, viral reactivation and genital ulcers did not significantly precede HIV acquisition. Rather, they occurred at seroconversion or post-seroconversion. Larger studies are needed to evaluate whether this is reactivation of HSV-2 due to a loss in immune control from HIV acquisition.

Laura Gerrick
Stevenson University
Mentor: Dr. Tonya J. Webb

Elucidating the role of CD1d-mediated antigen presentation on immune responses to cancer immunotherapy

It is estimated that cancer will be diagnosed in approximately 38.4% of all men and women, and is the second leading cause of death in the United States. Cancer immunotherapies such as immune checkpoint inhibitors (ICI) have completely revolutionized cancer care; however, ICI therapy has limited efficacy and only 20- 30% of patients respond to treatment. Therefore, understanding the genetic drivers in tumor cells that affect T-cell responses is essential. Our lab has previously shown that upregulation of the pro-survival factor, Bcl-xL, enhances natural killer T (NKT) cell responses to lymphoma; however, the mechanisms by which Bcl-xL regulates cancer immunogenicity remains unknown. In this study, we assessed protein expression in Bcl-xL overexpressing cell lines, compared to controls, by mass spectrometry and identified 36 differentially expressed. We utilized cBioPortal and probed the literature to determine if alterations in the differentially expressed genes had an effect on survival or ICI treatment efficacy. We hypothesized that the overexpressed genes would lead to better outcomes, due to increased ability of the immune system to recognize tumor cells, whereas the downregulated genes would correlate with poor outcomes. Increased expression in 3 of the 14 upregulated genes and decreased expression of 7 of the 22 downregulated genes led to significantly increased survival in diffuse large B-cell lymphoma, lower grade glioma, and/or skin cutaneous melanoma. Exploration of a subset of these genes in the literature provided a mechanistic link between cancer development and progression and the immune system. For example, RhoB knockout mice are more susceptible to tumor development, and in patients with glioma, RhoB upregulation led to significantly increased survival ($p= 0.0257$). Future studies will focus on modulating the expression of one or more of these genes *in vitro* using genetic manipulation and pharmacological inhibitors to determine their impact on cancer cell survival and the induction of anti-tumor immune responses. Collectively, our studies implicate a role for cancer-associated survival factors in dictating responsiveness to immunotherapy.

Gillian Blackwood
Loyola University Maryland
Mentor: Dr. Tonya Webb

The Impact of Ethnicity on Single Nucleotide Polymorphisms (SNPs) that Regulate Immune Signatures in Prostate Cancer

Nearly 1 in 9 men will be diagnosed with prostate cancer during his lifetime. It is estimated that African American men are 1.5 times more likely to be diagnosed with prostate cancer and twice as likely to die from this disease when compared to European American men. The disproportionality in diagnoses and mortality may be due to ethnicity-related genetic polymorphisms in immune-related genes. The goal of this study was to identify single nucleotide polymorphisms (SNPs) within co-signaling molecules that could result in differential responses to immunotherapy. In addition, we examined the impact of the SNPs on gene expression levels and investigated if these levels correlated with overall survival in prostate cancer. Six genes, CTLA4, CD28, CD40, CD40L, PD-1 and TNFSF4 (OX40L), were included in this study. A total of twenty-nine SNPs from these genes were investigated and have been associated with numerous diseases such as Systemic lupus erythematosus (SLE), Sickle Cell and Rheumatoid Arthritis. Among the twenty-nine SNPs studied, only two SNPs within CTLA4 were different between men of African American and European descent—the G allele of rs231778 and A allele of rs231772, both of which are only found in African American men that have been diagnosed with Rheumatoid Arthritis. Mutations in CTLA4, CD28 and CD40 have been reported to correlate with decreased survival in prostate cancer, thus our studies suggest that SNPs that result in downregulation or a loss of function in these proteins may impact cancer immune surveillance or responsiveness to cancer immunotherapy in prostate cancer patients.

Christina Kratzmeier
University of Maryland, College Park
Mentor: Dr. Tonya Webb

Impact of Ethnicity-Related SNPs in the Regulation of AMPK-mTOR Signaling in Prostate Cancer

Prostate cancer (PCa) is the second leading cause of cancer deaths among men in the US. Therefore, PCa is a significant health concern particularly in patients who are resistant to standard hormone therapies, such as androgen deprivation. These patients have developed castration-resistant prostate cancer (CRPC), which has a median survival rate of only 14-15 months. Outcomes for African American (AA) PCa patients are worse than European American (EA) PCa patients, and the mechanism underlying this cancer health disparity remains unknown. However, the metabolic AMPK-mTOR pathway is known to regulate PCa progression. New targeted drug treatments, such as mTOR inhibitors and AMPK activators, have been developed to target this pathway, but mTOR inhibitors have had little success in clinic. Studies from our lab have shown that AMPK activators differentially impact immune responses in EA and AA-derived PCa cell lines. We found that pretreatment of EA cell lines with AMPK activators, such as metformin, resulted in a 2-fold increase in natural killer T (NKT) cell responses; whereas, responses to AA cell lines remained unchanged. Therefore, we hypothesize that single nucleotide polymorphisms (SNPs) in the AMPK-mTOR pathway may contribute to the differences observed in treatment responsiveness. We conducted literature searches to identify SNPs in the AMPK-mTOR pathway. Then, we confirmed ethnicity-association through population allele frequencies reported in the database gnomAD. The majority of the SNPs identified had no associated clinical significance, thus we used various databases to denote the SNPs' locations to deduce functional impact. We identified 22 SNPs with higher allele frequencies in AA men that could dictate responsiveness to drugs targeting both AMPK and mTOR. 12/22 SNPs were located in the mTORC1 complex with the potential to interfere with mTOR inhibitors; therefore, alternative treatment strategies would be more effective for patients with these mutations. Our findings suggest that personalized approaches that include testing specific SNPs in PCa patients may help determine which patients would be more responsive to treatments targeting the AMPK-mTOR pathway.

Beza Ketema
University of Maryland College Park
Mentor: Dr. Paula Rosenblatt

Quality Improvement in Breast Cancer for African American Women

This project brings light to the disparities that exist within breast cancer treatment/survival rates and the characteristics of the cancer that plays into it. This is especially towards African American communities in comparison to the Caucasian community. There is a very evident gap in treatment availability and survival rate between these groups due to many factors such as poverty, geographic location, etc. Through patient chart evaluation, the characteristics and subtypes of breast cancer was recorded and investigated to see how different factors led to certain outcomes of the patient. This clinical study can have implications in the future to help researchers be more aware of the characteristics of breast cancer in African American women and differences they have compared to other races. Since black women have more variables hindering the improvement of their mortality rate, it's even more important that awareness is brought to this community.

Nicole Flegel
University of Maryland, College Park
Mentors: Drs. Mohummad Minhaj Siddiqui and Shu Wang

Impact of Radical Surgery vs. Radiotherapy on Prostate Cancer Specific Mortality and Overall Mortality in Men with Ductal Prostate Cancer

Prostate cancer is the second most common cancer in men worldwide and continues to be the second-leading cause of cancer death in men globally. Ductal adenocarcinoma of the prostate is a rare subtype of prostate cancer and is associated with delayed diagnosis and aggressive behavior similar to that of Gleason score 8-10 acinar adenocarcinoma. Because of its failure to increase prostate-specific antigen (PSA) levels and patients exhibiting normal digital rectal exams, ductal PCa often presents with advanced disease and less favorable prognosis. Efficacy of treatments for men with this rare histological subtype remain controversial. Using the Surveillance, Epidemiology, and End Results (SEER) program, we evaluated the impact of radical prostatectomy (RP) or external beam radiation therapy with/without brachytherapy (EBRT+/-BT) on Prostate Cancer-Specific Mortality (PCSM) and Overall Mortality (OM) for men with localized ductal PCa between the years of 2004-2016. Logistic regression was used to identify factors predicting RP treatment while Kaplan-Meier curves and cox regression were utilized for survival analysis. Our results revealed a 5-year-OS (overall survival) of 94.2% RP vs. 92.3% EBRT+/-BT ($p=0.708$) and a 5-year-DSS (disease-specific survival) of 96.7% RP vs. 92.3% EBRT+/-BT ($p=0.364$) with a mean follow-up time of 58 months. Survival analysis with KM-curves showed that patients receiving either radiotherapy or radical prostatectomy achieved similar OS and DSS (all $p>0.05$) leading us to the conclusion that radical surgery and radiotherapy prove to be equally effective treatments for men with this disease. Gleason score of 9 or higher and a PSA level between 10-20 ng/ml proved to act as independent risk factors for overall mortality while logistic regression revealed patients older than 75 or with a PSA>20 ng/ml were less likely to receive RP as treatment. Further analysis is being conducted to confirm this result in order to elucidate optimal treatment methods for patients with ductal PCa.

Sherry Fan
University of Maryland, College Park
Mentor: Dr. Charles Hong

Identification of Novel Genetic Susceptibility Loci for Thoracic and Abdominal Aortic Aneurysms Via Genome-Wide Association Studies of the UK Biobank

Aortic aneurysms are abnormal bulges that occur along the segment of aorta passing through the thoracic (thoracic aortic aneurysms, TAA) or abdominal cavities (abdominal aortic aneurysms, AAA), and can progress into life-threatening aortic dissections and ruptures. Family history is known to increase susceptibility for aortic aneurysms, but the specific genetic risk factors are not well understood. The UK Biobank is an ongoing prospective cohort study of over 500,000 UK participants ranging from 40-69 years of age at enrollment (between 2006-2010) and is a powerful tool for finding links between genetics and disease. We performed genome-wide association studies on the UK Biobank dataset to find novel variants associated with TAA and AAA. Individuals diagnosed with TAA (n=435) were age, sex, and ancestry matched to controls in a 1:20 ratio. Variants returned by the association study were filtered by p-value $<5x10^{-8}$ (threshold for genome-wide significance) and minor allele frequency >0.5%. Additional potential putative variants were identified using functional predictive scores (Eigen PC and Regulome DB), relevance of the affected genes, and p-value $<1x10^{-6}$ (suggestive threshold). The same analysis was performed for individuals diagnosed with AAA (n=1,363). Three independent loci, at the *CTNNA3*, *FRMD6*, and *MBP* genes, were identified for TAA. An additional locus at the *FBN1* gene within the suggestive threshold was highlighted for strong biological plausibility. Four independent significant loci, at the *LINC01021*, *ADAMTS8*, *ATOH8*, and *JAK2* genes, were found for AAA. Three additional loci meeting the suggestive threshold, at the *CDKN2B-AS1*, *CELSR2*, and *CDH9* genes, were highlighted for their biological plausibility. Overall, these genes are involved in cell-to-cell signaling and adhesion, as well as muscle cell differentiation, proliferation, and migration. The novel variants we identified for both TAA and AAA are candidates for genetic screening panels for those with familial aortic aneurysmal disease.

Sophie Nerone
Wheaton College
Mentor: Dr. Heather Ames

Identifying transcription factor markers of nuclear heterogeneity in glioblastoma

Glioblastoma (GBM) is an aggressive type of cancer that stems from glial progenitors found in the central nervous system (Mayo Clinic). GBM is the most common brain tumor in adults, accounting for 48% of all primary malignant brain tumors. Additionally, for the year 2020, an estimated 13,000+ Americans will receive a GBM diagnosis. While targeted therapies have become popular treatments for cancer, the primary modes of treatment for GBM are surgery, chemotherapy, and radiation. Due to the aggressiveness of the disease and limited treatment methods, the five-year survival rate for the disease is only 6% (National Brain Tumor Society). A better understanding of the mechanisms involved in GBM is needed to improve treatment, particularly the genetic and morphological heterogeneity that allows malignant cells to infiltrate diffusely in the brain. Previous research has suggested that transcriptional regulatory proteins can enable this phenotypic heterogeneity. The objective of this study is to identify biological pathways that can be clinically targeted for treatment of diffusely invasive tumor cells. Specifically, immunohistochemical stains for transcription factors used to pathologically identify glioblastoma will be analyzed for their association with abnormalities in nuclear morphology. A computational pipeline will be created to use the image analysis program CellProfiler and the Human Protein Atlas database to identify immunohistochemical correlates of nuclear heterogeneity in GBM.

Katrina Kuhn
University of Richmond
Mentor: Dr. Michal Zalzman

ChIP-Sequencing Analysis of ZSCAN4 Reveals its Chromatin and Gene Targets

Cancer cells and embryonic stem (ES) cells share an unlimited capacity for self-renewal as well as many gene expression networks. The "stemness" state in ES cells is maintained by the core pluripotency factors OCT3/4, NANOG and SOX2. Previous studies have demonstrated that, similar to ES cells, cancer cells can harness these factors for their survival, inhibition of differentiation, and self-renewal. In this work, we study the early embryonic factor Zinc finger and SCAN domain containing 4 (ZSCAN4) which has been previously shown to promote telomere extension and developmental potency. We recently reported that a short induction of ZSCAN4 increases cancer stem cell (CSC) frequency in head and neck squamous cell carcinoma (HNSCC) and leads to upregulation of pluripotency and CSC factors. Importantly, we showed that ZSCAN4 alters the chromatin state and the epigenetic profile of HNSCC cells. Our data show that ZSCAN4 leads to a functional histone 3 hyperacetylation at the promoters of the core pluripotency factors, which leads to an upregulation of CSC factors. Based on this data, we hypothesized that ZSCAN4 facilitates chromatin remodeling, which induces CSC factors. Therefore, we used chromatin immunoprecipitation followed by next generation sequencing (ChIP-Seq) to identify the genome-wide binding sites of ZSCAN4 in HNSCC cells. Our data indicate that the human ZSCAN4 binds to over 70,000 sites and 8,900 unique genes. Additionally, 68% of those sites are within genes and promoters. Importantly, our data establish ZSCAN4 as new factor regulating telomeric chromatin and comprehensively identify its target genes.

Ilana Moffet
University of Michigan – Ann Arbor
Mentor: Dr. Cheryl Knott

Antioxidants as an Adjuvant Therapy in Cancer Patients undergoing Chemotherapy with a Curative Intent

Conventional cancer therapies such as chemotherapy and radiation can cause the patient to experience adverse side effects and can negatively impact quality of life for the patient. For these reasons, patients often turn to alternative therapies to help manage these effects. These therapies are not necessarily prescribed by a doctor. One common therapy used by patients is antioxidant supplementation or dietary change to introduce more antioxidants into the body. The issue with alternative therapies like antioxidants is that there is insufficient scientific research available about their safety and efficacy and thus physicians are not in a position to advise their patients on use of alternative therapies. In order to learn more about antioxidants and other alternative medicine, a comprehensive online literature review was conducted and over 50 articles were collected and summarized into a table. The review was focused on antioxidants and articles pertaining to antioxidant use as an adjuvant therapy to chemotherapy. The most popular antioxidants used by cancer patients undergoing chemo- or radiotherapy with a curative intent were vitamin C and selenium. Very few articles reported the antioxidants having a negative interaction with the chemotherapy or radiation that the patient was receiving. There was an overall positive effect by the antioxidants on the adverse side effects of conventional medicine and improvement of quality of life. Research in this area is limited by small sample sizes and thus more trials are needed with larger groups of patients. In order to have scientifically backed findings that physicians can use in consultation with patients, additional research is warranted.

Arta Kasaeian
University of California, Los Angeles
Mentors: Drs. Ashkan Emadi and Farin Kamangar

Targeting Serine Metabolism in combination with Glutamine Depletion for Acute Myeloid Leukemia

Acute Myeloid Leukemia (AML) is an aggressive form of cancer with a high mortality rate. Standard of care has only changed minimally over the last several decades, underscoring the need for new therapeutic approaches. Cancer cell metabolic reprogramming results in elevated nutritional needs which can then be exploited to hinder their proliferation through. Glutamine depletion has emerged as a therapeutic approach for AML, which has demonstrated a dependence on glutamine for survival. Asparaginase, an enzyme that hydrolyzes asparagine and glutamine to aspartate and glutamate, respectively, can be used to directly reduce glutamine availability. In preliminary studies using the pegylated recombinant asparaginase Pegcrisantaspase (PegC) in a mouse model of AML, we found that PegC treatment completely depleted glutamine and asparagine from the plasma and significantly reduced tumor burden. Interestingly, we also observed an increase in plasma serine levels following PegC treatment. The serine biosynthesis pathway is essential for numerous cancer cell functions, including protein and nucleic acid synthesis and maintaining cellular redox state, and others have shown that in response to glutamine deprivation, leukemia cells upregulate key enzymes in the serine biosynthesis pathway, suggesting that serine upregulation may be a targetable compensatory mechanism. Inhibitors of D-type cyclins (D3) and their cyclin-dependent kinase CDK6 can lower the flow of glycolytic intermediates into the serine pathway. Therefore, we hypothesize that targeting serine biosynthesis indirectly using D3-CDK6 inhibitors will synergize with glutamine depletion by PegC to induce AML cell death. To test this, we propose to treat a panel of human AML cell lines and primary patient samples with CDK6 inhibitors alone and in combination with PegC and assess cell proliferation and drug synergy. The impact of D3-CDK6 inhibitors on serine biosynthesis will also be determined by examining the expression and function of key pathway enzymes. At the conclusion of our studies, we expect to discover new mechanistic insights into glutamine depletion in AML and determine the potential of CDK6 inhibition combined with glutamine depletion as a novel therapeutic strategy.

Thomas Hill
La Salle University
Mentor: Dr. Helen Dooley

Identifying Complement Proteins in Sharks: The Oldest Ancestor of the Modern Immune System

The complement system is a branch of the innate immune system that serves as one of the first lines of defense against invading pathogens. Through a cascade of protein-protein interactions it mediates opsonization, inflammation, and cell destruction. Comprehensive studies of the mammalian complement system have identified specific pathways and molecules involved in its execution. Through this understanding, new questions have emerged regarding how such a complex system evolved into its present state. This work focuses on mapping the evolution of the complement system by identifying complement molecules within different shark species, the most ancient vertebrate lineage to have components of both adaptive and innate immunity. We examined the presence or absence of complement molecules in the whale shark and the brown-banded bamboo shark. By determining the composition of complement molecules in these species of sharks, we can gain insight into how this system has changed over evolutionary time. Using the NCBI database, amino acid sequences of known complement molecules in humans were catalogued. These sequences were then used to identify potential orthologs in whale and bamboo shark using the BLAST algorithm against a verified shark transcriptome database: Squalomix. The resulting protein hits were aligned with those sampled from key vertebrate species using the MAFT and PRANK algorithms. The resulting alignments allowed for the creation of phylogenetic trees. By analyzing these trees, the similarities and differences between the composition of complement molecules in sharks and humans was determined. The results of this analysis will be presented. This work is an important foundation for *in vivo* studies in sharks aimed at understanding the functions of the complement system in this group.

Samantha Rea
Stevenson University
Mentor: Dr. Paul Shapiro

Protein Expression Patterns in Monolayer and spheroid Melanoma Cell cultures with Acquired Resistance to BRaf and MEK1/2 Inhibitors

Approximately 50% of malignant melanomas contain a Braf activating mutation, which promotes proliferation, Though the current standard treatment of BRaf/MEK inhibitor combination therapy targets the mutated kinase inhibitors, eventually resistance develops through both ERK1/2 independent and dependent activation, limiting sustainability of treatment. Many studies observing the changes leading to resistance in cancer models choose to exclusively study either a monolayer or 3D spheroid cell model, however no studies currently compare the differences in protein expression of each. To generate an understanding of the limitations and variation between each model, a comparison of protein and canonical pathway expression was completed using BRaf mutated resistant and nonresistant A375 melanoma cells in monolayer and 3D spheroid models. Due to the limited access of nutrients of the inner cells in the spheroid model, metabolic pathways were hypothesized to have observable changes in expression, whereas changes in metastatic pathways were predicted to be changed in the monolayer model cells. Cells resistant to PLX4032/AZD624 combination therapy were generated through incubation in the presence of the drugs, while nonresistance cells were incubated in their absence. Each cell type was grown as both a monolayer 3D spheroid. Mass spectrometry and Quiagen Ingenuity database were used to identify and quantify the proteins and pathway expression. As expected, upregulated pathways in the spheroid were more associated with control of metabolic pathways including oxidative phosphorylation, while the monolayer upregulated pathways associated with metastasis including TGF-β signaling. These observations emphasize the differences in both monolayer and spheroid models and may provide possible future therapeutic targets to overcome protein kinase inhibitor resistance.

Bria Thomas
Loyola University of Maryland
Mentor: Dr. Christabel Cheung

Measuring Financial Toxicity of Cancer in Young Active-Duty Military Patients

When faced with a cancer diagnosis, adolescent and young adult (AYA) active-duty patients in the military (diagnosed between ages 15-39 years) are faced with potential implications for their career paths, families, and financial standing. Existing literature suggests that financial well-being (FWB) measures can explain socioeconomic conditions outside of the traditional measures – like education and career. However, it is undetermined if these measures can describe the "financial toxicity" experienced by AYA military cancer patients. Currently, most investigations are centered around measures of FWB concerning non-military populations. AYA military cancer patients experience many benefits like medical coverage on cancer drugs, treatment, and the continuation of base salary. On the other hand, clinical experience indicates that during cancer treatment, increasing out-of-pocket expenses and lost-opportunity costs for young military patients and family members make this patient care population vulnerable to financial toxicity. The objective of this study was to develop a measure of financial well-being to assess financial toxicity in the military AYA cancer patient population. The investigator team conducted focus groups and key informant interviews (n=24) with active-duty AYA cancer patients, their spouses, cancer care providers, and military unit commanders at Tripler Army Medical Center and Schofield Barracks in Honolulu, HI. Findings revealed that the experiences of AYA active-duty military patients regarding financial toxicity, after cancer diagnosis, can be sorted into material, psychosocial, and behavioral domains to describe the FWB of this population. Distinguishing the financial toxicity of the military AYA cancer patient population into these domains inform the development of interventions and warrants that those efforts are informed by an aspect of FWB most impacted by military cancer care.

Angela Sun
University of Maryland, College Park
Mentor: Dr. Tami Kingsbury

Probing the SIX proximal interactome for functional protein interactions

The PAX-SIX-EYA-DACH network (PSEDN) is a conserved network of transcription factors crucial for development. Misexpression of these factors has been associated with multiple hallmarks of cancer. Our lab has shown that overexpression of SIX1 or SIX2, PSEDN members, induces differentiation in human TF1 erythroleukemia cells *in vitro*, and that this response is dependent on expression of the well-known hematopoietic regulator GATA1. It is not known what other proteins may mediate this SIX-GATA1 interaction, nor how interactions between the PSEDN and other transcriptional networks like the GATA1 network, may play a role in various other disease phenotypes. To further explore this, our lab performed a BioID screen to determine proteins that associate with SIX1 and SIX2 in TF1 cells, generating a list of 675 potential SIX interactors. The goal of this project was to identify and prioritize candidates from this list for functional protein interaction. To identify potential transcription factors cooperating with SIX proteins, Gene Set Enrichment Analysis (GSEA) was performed on RNAseq data from SIX1 overexpressing vs control TF1 cells. This analysis yielded a panel of 87 transcription factors whose target genes were differentially expressed in response to SIX1 overexpression. This panel was then compared to our BioID SIX proximal interactome to prioritize functionally relevant SIX interacting factors, resulting in a more focused list of 12 proteins, including GATA1. Finally, mining through genomic databases such as cBioPortal and OASIS revealed that several of these proteins have existing functional implications in phenotypes similar to SIX: ZNF589, for example, regulates cell viability in hematopoietic stem progenitor cells and is important for erythropoietic differentiation. These proteins will be our top candidates for future gain-of-function and loss-of-function studies, with the eventual goal of identifying functional SIX-protein interactions which serve as drug targets for the diseases in which they are implicated.