

The OSR Student Research Forum: 2021 Nathan Schnaper Intern Program in Translational Cancer Research and ACS Diversity in Cancer Research Program



July 30, 2021
Health Science Facility-II Auditorium

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-AND-

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

OSR Student Research Forum: 2021 NSIP Research Symposium

Friday, July 30, 2021
9:00 am to 4:35 pm
HSFII Auditorium

	Speaker	Mentor
9:00 to 9:20 am	Breakfast	
9:20 to 9:25 am	Bret A. Hassel, PhD <i>Director's Welcome</i>	
9:25 to 9:37 am	Beza Ketema <i>Exploring Neuromuscular Mechanisms in Typically Developing Healthy Children and Adolescents</i>	Victoria Marchese
9:37 to 9:49 am	Arta Kasaeian <i>Targeting Serine Metabolism in Combination with Glutamine Depletion for Acute Myeloid Leukemia</i>	Ashkan Emadi
9:49 to 10:01 am	James Hsia <i>Enterovirus D68 and Poliovirus Infections are Lytic instead of Persistent in K562 Cells differentiated by Hydroxyurea and Sodium Butyrate</i>	William Jackson
10:01 to 10:13 am	Samantha Frias <i>Investigating the Exosomes Secreted by Cells expressing the RTA gene of HHV-8</i>	Alfredo Garzino-Demo
10:13 to 10:25 am	Ethan Fuller <i>SMC5/6-dependent SUMOylation of PML is Involved in Inhibition of HBV cccDNA Activity</i>	Lishan Su
10:25 to 10:37 am	Skylar Chan <i>Identification of the Activity of Enhancers and Promoters Specific for HPV-related Head and Neck Squamous Cell Carcinoma</i>	Daria Gaykalova
10:37 to 10:55 am	Coffee Break	
10:55 to 11:07 am	Gillian Blackwood <i>The Role of Monoglycerol Lipase on NKT Cell Responses to Lymphoma</i>	Tonya Webb
11:07 to 11:19 am	Jordyn Best <i>The Impact of Monoglyceride Lipase Deficiency on Natural Killer T Cell Activation</i>	Tonya Webb

11:19 to 11:31 am	Elshaday Behailu <i>The Role of Granzyme B on the Migration of Various Cancer Cell Lines</i>	Xuefang Cao
11:31 to 11:43 am	Nicole Flegel <i>Short-Circuiting Tumor Tolerance: Targeting SHP-1 to Improve T cell Activation</i>	Nevil Singh
11:43 to 11:55 am	Maanasa Gurram <i>Development of Patient-Derived Organoids for Use in Risk Stratification and Treatment Optimization of Bladder Cancer</i>	Minhaj Siddiqui
11:55 to 1:00 pm	Lunch <i>HSFII 4th floor rms 400 and 431</i>	
1:00 to 1:12 pm	Anusha Bhat <i>Investigating the Role of S100B Protein in the Metastasis of Melanoma</i>	Kaylin Adipietro
1:12 to 1:24 pm	Sasha Singh <i>Role of Angiopoietin-like 4 in Head and Neck Squamous Cell Carcinoma Metastasis</i>	Silvia Montaner
1:24 to 1:36 pm	Alexia Smith <i>Impact of G Protein-Coupled Receptor 68 (GPR68) Inhibition on Glioblastoma Survival</i>	Charles Hong
1:36 to 1:48 pm	Tobias Liska <i>MBNL1 Mediated Alternative Splicing of Genes Associated with Nervous System Development in Glioblastoma Multiforme</i>	Eli Bar
1:48 to 2:00 pm	Corinne Martin <i>Impact of RPTOR mutations on axon projection, correlation to brain cancer progression</i>	Alex Pouloupoulos
2:00 to 2:15 pm	Coffee Break	
2:15 to 2:27 pm	Jennifer Yan <i>Purification and Analysis of an Fn14 Monoclonal Antibody and its Antigen Binding Fab Fragment as Targeted Nanoparticle Cancer Therapy</i>	Jeffrey Winkles
2:27 to 2:39 pm	Samantha Rea <i>Induction of CD8+ T cell-mediated Cancer Cell Lysis via Antigen-Polymer Conjugated Nanoparticles</i>	Ryan Pearson

2:39 to 2:51 pm	Kristen Zaranski <i>The effect of Doxycycline and Celecoxib on the Growth and Metastasis of HT-1080 Fibrosarcoma and U-2OS Osteosarcoma Cell Lines</i>	Rena Lapidus
2:51 to 3:09 pm	Jameila Barrett and Demi Morris <i>Investigating Vulnerabilities of Mitogen Activated Protein Kinases in Diffuse Large B-cell Lymphoma</i>	Paul Shapiro
3:09 to 3:21 pm	Alyssa Low <i>A Non-Canonical Mechanism for the Antiproliferative Activity of Tristetraprolin</i>	Gerald Wilson
3:21 to 3:35 pm	Coffee Break	
3:35 to 3:47 pm	Sophie Liu <i>The effect of ZSCAN4 on Histone Modifications and Cell Stemness</i>	Michal Zalzman
3:47 to 3:59 pm	Sara Jain <i>The Effect of iCRISPR/Cas9 Knockout of ZSCAN4 on Cancer Stem Cell Factors</i>	Michal Zalzman
3:59 to 4:11 pm	Ayooluwakiitan Oluwafemi <i>Early Predictive Marker of Breast Cancer Metastasis</i>	Nikki Walker
4:11 to 4:23 pm	Alexandra Jerrett <i>Discovery of ROS-induced Tubulin Detyrosination and Acetylation in Breast Cells</i>	Stuart Martin
4:23 to 4:35 pm	Lena King <i>Factors Associated with Cytotoxic Potential in Neonatal Vd2 Cells</i>	Cristiana Cairo
4:35 pm	Bret Hassel <i>Closing Remarks</i>	

Abstracts

(in speaking order)

Beza Ketema

University of Maryland, College Park

Mentor: Dr. Victoria Marchese

Exploring Neuromuscular Mechanisms in Typically Developing Healthy Children and Adolescents

The mechanisms involved in physical function limitations in children with various health conditions are not fully understood, which leaves uncertainty in designing interventions to address functional limitations. By studying the many characteristics that may be involved such as muscle strength, rate of muscle activation, balance, exercise capacity, pain and quality of life in 10 healthy children, researchers can gain a better understanding of how to measure physical function and be able to evaluate the effectiveness of treatment interventions for children with limitations. For example, the intra- and inter-reliability for measuring diaphragm thickness measured through B-mode ultrasound was good to excellent. This shows that the measurements taken for diaphragm thickness is reliable for healthy children. Knowing that this is a reliable measurement, this method can be very useful for clinical research and practice. In the future, the psychometrics of other neuromuscular mechanisms will continue to be studied and will lead to improved approaches to evaluating the effectiveness of intervention strategies that could improve these impairments.

Arta Kasaeian

University of California, Los Angeles

Mentor: Dr. Ashkan Emadi

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. Glutamine depletion has emerged as a therapeutic approach for AML, which depends 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, significantly reducing tumor burden but also observed an increase in plasma serine levels. 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 cyclin-dependent kinase 6 (CDK6) have been shown to lower the flow of glycolytic intermediates into the serine pathway, therefore we hypothesize that targeting serine biosynthesis indirectly using CDK6 inhibitors will synergize with glutamine depletion by PegC to induce AML cell death. To test this, we treated the human AML cell lines, MV411 and MonoMac6, with the CDK6 inhibitors palbociclib and ribociclib, alone and in combination with PegC and assessed cell proliferation and drug synergy. We found that both ribociclib and palbociclib synergized with PegC to inhibit cancer cell proliferation in the AML cells lines tested. While it is not clear yet whether the observed synergism is due to an impact on serine biosynthesis, our results point to the potential of CDK6 inhibition combined with glutamine depletion as a novel therapeutic strategy for AML.

James Hsia
Howard University
Mentor: Dr. William Jackson

Enterovirus D68 and Poliovirus Infections are Lytic instead of Persistent in K562 Cells differentiated by Hydroxyurea and Sodium Butyrate

Enterovirus D68 (EV-D68) is a respiratory virus with a genome very similar to poliovirus. Poliovirus (PV) caused 15,000 cases of paralysis (poliomyelitis) each year in the United States before vaccines were available. EV-D68 is also associated with paralysis disease, in particular a rare form of childhood paralysis called acute flaccid myelitis. While these infections are normally self-limiting, the virus has been shown to cause rare long-term persistent infections in some individuals. In cell culture models, picornaviruses are normally lytic and cause significant cell death. However, PV and EV-D68 infection of an immortalized chronic myelogenous leukemia human cell line, K562, results in a persistent infection with a large majority of cells remaining viable. K562 cells are a multipotent cell line that can differentiate into more specialized cell lineages. It had been shown that hemin-differentiated cells can succumb to lytic infection. It was unknown whether any other methods of differentiation would cause a similar effect, but we hypothesize that differentiation into erythroblastoid cells with hydroxyurea and sodium butyrate will change infection of K562 cells from persistent to lytic. K562 cells were successfully differentiated by hydroxyurea, sodium butyrate, and phorbol 12-myristate 13-acetate (PMA) followed by infection with either PV or EV-D68. Percent viability was taken at 0, 24, and 48 hours post infection with trypan blue or tetrazolium staining. Cell viability after infection significantly decreases for cells treated with hydroxyurea and sodium butyrate compared to infected, undifferentiated cells. Western blotting of samples demonstrated that treated cells display several differentiation markers as well as markers of infection. This data suggests that differentiation by hydroxyurea and sodium butyrate causes K562 cells to become susceptible to lytic infection, and further study with flow cytometry and viral titrations may offer more insight into the mechanisms of persistent infection compared to lytic infection.

Samantha Frias
Towson University
Mentor: Dr. Alfredo Garzino-Demo

Investigating the Exosomes Secreted by Cells expressing the RTA gene of HHV-8

HHV-8, or Kaposi's sarcoma-associated herpesvirus, is an oncogenic virus that has been directly implicated in several cancers including Kaposi's sarcoma (KS) and a type of lymphoma. The exact oncogenesis behind T-cells affected by HHV-8 possibly transforming into B-cell cancers are not yet understood and may include modification of the target cells or of the microenvironment via exosomes. Exosomes are extracellular vesicles released by multivesicular body (MVB) fusion with the cell surface that carry nucleic acids, lipids, and proteins from the parent cell to the target cell. Preliminary evidence shows that exosomes can affect the target cell's phenotype and cell behavior. We are investigating if exosomes contribute to the effect of HHV-8 on target cells and/or the microenvironment to favor cancer development. Our experiment adopted a simple, infection-free system based on the expression of RTA, a key gene of HHV-8. We isolated and purified exosomes from a cell line that can be induced to express RTA using a specialized kit and through ultracentrifugation. To ensure that the quality of the sample is appropriate, we performed western blots for tetraspanins common in exosomes: CD9, CD63, and CD81. To differentiate between exosomes and microvesicles, which are found on the plasma membrane, we also tested for the protein ARF6 which are specifically found on the plasma membrane. We hope to visualize bands in any exosomal tetraspanins then analyze the RNA and protein contents through RNA global analyses and proteomics. Subsequently, we plan to incubate exosomes with target cells to evaluate changes in global expression patterns in the target cells, to determine the role of exosomes in favoring cancer development. Looking forward, these changes will shed light on the potential mechanisms in target cells that contribute to its transformation and further research can be conducted.

Ethan Fuller
Gonzaga University
Mentor: Dr. Lishan Su

SMC5/6-dependent SUMOylation of PML is Involved in Inhibition of HBV cccDNA Activity

Chronic hepatitis B virus (HBV) infection is a major risk for the development of hepatocellular carcinoma (HCC). Despite current success in prophylactic Hepatitis B (HBV) vaccination, HBV remains a public health burden with an estimated 300 million people chronically infected. Failure to cure chronic HBV infection is primarily due to the resistance of the HBV covalently closed circular DNA (cccDNA) genome to current HBV therapies. Thus, an understanding of the mechanisms that lead to cccDNA stability represents an essential goal. Recently, our group and others have identified Structural Maintenance of Chromosomes (SMC) 5/6 protein complex as a host factor that represses HBV cccDNA transcription, which is countered via targeted degradation of SMC5/6 by HBV protein X (HBx). SMC5/6 was additionally shown to colocalize with Promyelocytic Leukemia (PML) protein bodies, which are known to be involved in a variety of cellular homeostatic pathways including host antiviral defense. Components of SMC5/6 show SUMO-ligase activity, and PML bodies require SUMOylation to carry out their antiviral defense. Notably, we have discovered the interferon-stimulated PML gene also inhibits HBV cccDNA activity. However, the mechanism by which PML initiates this response remains unclear. To investigate the role of PML and HBx-SMC5/6 in HBV infection, HBV reporter genes expressing Gaussia Luciferase (GLuc) were co-transfected with various PML isoforms, and HBV transcriptional activity was measured. In 293T and HepG2 cell lines co-transfected with SMC5/6, PML, and SUMO, we observed by immunoprecipitation (IP) that SMC5/6 interacts with PML complex and increases PML SUMOylation, indicating a putative contribution of PML SUMOylation by SMC5/6 to aid in HBV cccDNA suppression. Taken together, our findings provide support for the idea that SMC5/6 contributes to PML sumoylation in the repression of HBV replication and provide insight into new therapeutic approaches for an HBV cure.

Skylar Chan
University of Maryland, College Park
Mentor: Dr. Daria Gaykalova

Identification of the activity of enhancers and promoters specific for HPV-related head and neck squamous cell carcinoma

Human papillomavirus (HPV) infection increases the risk of head and neck squamous cell carcinoma (HNSCC), which comprises cancers of the mucosal surface of the oral cavity, pharynx, hypopharynx, and larynx. It is the 6th most common cancer worldwide, with 890,000 new cases and 450,000 deaths in 2018. HPV-positive HNSCC and HPV-negative HNSCC both have pervasive gene expression alteration. HPV-positive HNSCC has fewer genetic alterations than HPV-negative HNSCCs, implying a greater range of epigenetic changes in HPV-positive HNSCC to explain the load of transcriptomic changes. Previous research sequenced DNA bound by H3K27ac histones from HPV-positive HNSCC and non-cancer controls using chromatin immunoprecipitation sequencing (ChIP-seq) to identify active promoters, enhancers, and super-enhancers in cancer and healthy tissues. We extend these results by evaluating the expression of enhancer RNA (eRNA), noncoding RNAs at the enhancer/promoter regions/domains identified by ChIP-seq data analysis. It has been suggested that eRNAs stabilize the promoter-enhancer binding, which boosts transcription. We extracted eRNAs from non-cancer uvula samples from healthy patients, HPV-positive HNSCC samples expanded in mice, and HPV-positive HNSCC cell lines derived from patient samples. We performed RNA transcript quantification on the eRNA data and compared the eRNA differential expression. We also computed changes in the regulation of the Molecular Signatures Database (MSigDB) hallmark pathways in the eRNA data using gene-set enrichment analysis on the domains. We identified various up and down-regulated genes and pathways in cancer-specific enhancer domains. Our project established concordance between ChIPseq histone modification data and eRNA data, and identified high-impact genes and pathways relevant to HPV-positive HNSCC. Future studies will define the most dysregulated pathways in cancer-specific promoters, enhancers, and super-enhancers; and validate results using data from The Cancer Genome Atlas (TCGA).

Gillian Blackwood
Loyola University Maryland
Mentor: Dr. Tonya Webb

The role of monoglycerol lipase on NKT cell responses to lymphoma

The incidence of lymphoma in the US is rising. Clinical studies have demonstrated that while immunotherapy can effectively treat lymphoma early, many patients relapse resulting in 20,000 US deaths each year. Thus, new treatment strategies are urgently needed. It has been well established that natural killer T (NKT) cells play a critical role in the host's anti-tumor immune response; however, the mechanisms by which NKT cells recognize cancer cells remain unclear. Previous studies from our lab have shown that alterations in cellular metabolism result in increased CD1d-mediated NKT cell activation. Based on a proteomic screen, we identified monoglyceride lipase (MGL) as a potential factor involved in anti-tumor responses mediated by NKT cells. To test this hypothesis that MGL regulates CD1d-mediated antigen presentation to NKT cells, we utilized MGL deficient (MGLKO) animals. We examined NKT cell activation in response to stimulation by splenocytes isolated from wild type, MGL hemizygous, and MGLKO mice. We observed minimal cytokine production by NKT cells following stimulation with splenocytes from each group. In addition, general stimulation of the endogenous population of T cells with anti-CD3/CD28 microbeads failed to induce a robust response. Future studies will focus on characterizing the immune response in MGL deficient mice in order to gain a better understanding of its role in cancer immune surveillance.

Jordyn Best
Stevenson University
ACS-Diversity in Cancer Research Intern
Mentor: Dr. Tonya Webb

The Impact of Monoglyceride Lipase Deficiency on Natural Killer T Cell Activation

Cancer is a significant health issue worldwide and is the second leading cause of death in the United States (US). According to the American Cancer Society, it is estimated that in 2021, 1,898,160 new cancer cases and 608,570 cancer deaths will occur in the US. Immunotherapy is a method of treatment that utilizes a person's own immune system to detect and fight cancer. Natural killer T (NKT) cells are immune cells that have characteristics of both the innate and adaptive arms of the immune system, specifically natural killer (NK) cells and T cells, bridging these two parts of the immune system. Studies investigating NKT cell-based immunotherapy have shown limited efficacy. Therefore, our research is focused investigating cancer-associated factors that impact NKT cell activation. Previous research has shown that stimulation of NKT cells with cells in which monoglyceride lipase (MGL) has been downregulated or inhibited results in high levels of cytokine production, compared to controls. In addition, MGL deficient (MGLKO) mice are highly susceptible to developing tumors. We hypothesize that MGL regulates NKT cell development and function, leading to an increase in susceptibility to cancer. In these studies, we used MGL wildtype, hemizygous, and MGLKO mice to investigate NKT cell phenotype and function, as assessed by flow cytometry and ELISA. Our preliminary studies suggest that NKT cells are generally unresponsive to the thymocytes of all three MGL groups. This may indicate an underlying issue of the immune system. Collectively, these studies will enhance our understanding of NKT cell biology and may be used to help develop better NKT cell-based therapies for the treatment of cancer.

Elshaday Behailu
University of Maryland Baltimore County
Mentor: Dr. Xuefang Cao

The Role of Granzyme B on the Migration of Various Cancer Cell lines

Granzyme b is a protease commonly found in the apoptotic pathway. When paired with perforin, a glycoprotein that creates pores in target cells, they cooperatively target specific cells for apoptosis. Our study focused on the effect of granzyme b on cancer cells without the inductively targeting function of Perforin. We hypothesized that granzyme b may aid cancer cells with their permeability and spread to the extracellular matrix of cells. Our first step was to lyse a set of b-16 cancer cells and perform an RT-PCR in order to approximate their RNA concentration. Our plan of action was to lyse a set of B-16 cancer cells, pair them with granzyme b protein and test their permeability across a gel media. We set up a control b-16 sample as well without the granzyme b addition. Our results were in congruence with the thought process that granzyme b may aid the movement of cancer cells when not paired with Perforin. We also analyzed the effect of different cell lines on the vitality of mice *in vivo*. We injected several cancer cell lines into the pelvic area of mice, intramuscularly and monitored their vital signs including loss of hair, diarrhea, weight loss and general activity over the course of 5 weeks.

Nicole Flegel
University of Maryland, College Park
Mentor: Dr. Nevil Singh

Short-Circuiting Tumor Tolerance: Targeting SHP-1 to Improve T cell Activation

Within our adaptive immune system, there is a delicate balance between providing protection to the body from foreign pathogens and preventing autoimmunity. This balance between T cell immunity and tolerance is also reflected in the positive and negative regulatory mechanisms that control T cell activation, mainly through the interactions of protein tyrosine kinases (PTKs) and phosphatases (PTPs). Cancers often evade T cell responses by engaging inhibitory receptors such as PD-1, CTLA4, TIM-3 etc. Many of these can be targeted for immunotherapeutic treatments, but due to their redundancy treatments can often lead to resistance. Since several of these checkpoints inhibit T cell activation via their signaling using PTPs, we hypothesized that directly targeting cellular PTPs to increase the ratio of intracellular positive to negative activation signals may enhance the antitumor response of effector T cells. To investigate this, we first targeted the phosphatase SHP-1. Dominant negative protein mutants of SHP-1 were constructed through molecular cloning in order to trap substrate proteins of our PTP and inhibit further downstream signaling. These dominant negative SHP-1 genes were subsequently cloned into a murine retroviral vector for expression studies. Transfection of a cell line and western blotting were used to verify the expression of the SHP-1 dominant negative proteins within mammalian cells. Expression studies within primary T cells are currently being run in order to observe the effect of inhibiting SHP-1 activity on the activation of T cells *in vitro*. Once validated, these signaling constructs can be introduced into CAR-T or adoptive-therapy T cells to enhance their anti-tumor function.

Maanasa Gurram
University of Maryland College Park
Mentor: Dr. Mohummad Minhaj Siddiqui

Development of Patient-Derived Organoids for Use in Risk Stratification and Treatment Optimization of Bladder Cancer

Bladder cancer is the 4th and 9th most common cancer in males and females respectively, causing significant morbidity and mortality. The approach to treatment and potential benefit of neoadjuvant chemotherapy (NAC) prior to cystectomy varies amongst patients. Thus, the ability to predict patient response to NAC based on tumor classification holds the potential to personalize patient treatment and improve the outcome. The preliminary task prior to conducting these studies is the development of organoids, which serve as 3D models that more accurately represent the molecular pathology of the parental tumor and behave in a genomic manner that is consistent with tumor evolution in culture. Our lab tested different methodologies to optimize the development of organoids through patient-derived tissue samples. The in vivo environment was mimicked to effectively establish and grow the organoids. We successfully established four organoid lines, two derived from cancerous bladder tissue, one derived from normal bladder tissue, and one derived from a T24 cell line using the Cancer Tissue-Originated Spheroid (CTOS) method. We performed microscopic imaging and observed organoid survival and consistent growth through the span of 10 days, with imaging done on Day 1, Day 5, and Day 10. Future treatments can be performed on the developed organoids to determine if there is an association between metabolic profiles of a tumor and its molecular subtype and if this association can predict the tumor's therapeutic sensitivity to chemotherapy. The organoids will additionally be used for orthotopically implanted xenografts in mice and imaging of the xenografts before and after chemotherapy will be studied to determine the effectiveness of Hyperpolarized ¹³C MRI in identifying tumor behavior and treatment sensitivity through the metabolomic phenotype. Future directions include the initiation of a clinical trial focused on translating hyperpolarized [1-¹³C]pyruvate MRI as a human application for bladder cancer imaging.

Anusha Bhat
Carnegie Mellon University
Mentors: Drs. Kaylin Adipietro & David Weber

Investigating the Role of S100B Protein in the Metastasis of Melanoma

Melanoma is the 5th most common type of cancer, representing an estimated 5.6% of new cancer cases in the United States in 2021. While malignant melanoma (MM) only accounts for approximately 1% of all skin cancers, it is the most aggressive and deadliest form of this cancer subtype. The S100B calcium-binding protein is a marker for malignant melanoma, with elevated levels indicating a poor prognosis, disease recurrence, high metastatic rate, and increased mortality. S100B contributes to metastatic disease progression through multiple mechanisms involving p53, cytokine secretion, and the MAPK signaling. The MAPK pathway exhibits increased activity in melanoma cells, due to the presence of an upstream activating mutation in BRAF (V600E/D) that impacts proliferation, differentiation, migration, survival, and overall tumor progression. S100B was previously shown to bind RSK (90 kDa Ribosomal S6 Kinase), which is a downstream effector in MAPK signaling. This prevents RSK from becoming phosphorylated and inhibits translocation of it to the nucleus, thus localizing it to the cytoplasm. Based on this interaction between the signaling pathway and the S100B protein, we used the human melanoma cell line WM115 (which expresses high levels of S100B) to characterize the impacts of S100B on the migratory capacity and composition of the extracellular matrix. Our data suggest that the elimination of S100B slows the rate of migration and alters the expression of proteins associated with metastasis.

Sasha Singh
Stevenson University
ACS-Diversity in Cancer Research Intern
Mentor: Dr. Silvia Montaner

Role of Angiopoietin-like 4 in Head and Neck Squamous Cell Carcinoma Metastasis

Head and neck squamous cell carcinomas (HNSCCs) are a diverse group of cancers arising from the mucosal lining of the oral cavity, pharynx, and larynx, that remain the 6th most prevalent cancer worldwide. Local lymph node metastasis leads to HNSCC poor prognosis and has a decreased survival rate because of the likelihood of further development of distant metastases. Unfortunately, 50% of HNSCC present locoregional metastasis at the time of diagnosis and current treatment for these patients are limited.

HNSCC cells can escape apoptosis, migrate, invade, and promote angiogenesis. Contrary to traditional chemotherapy treatments, epithelial growth factor receptor (EGFR) antibodies (used to treat HNSCC) target the downstream signaling pathways that induce angiogenesis and cause tumor proliferation. However, they have limited success. Interestingly, ANGPTL4, an angiogenic factor that induces cell migration, invasion, and vessel hyperpermeability is upregulated in most human cancers, including oral cancer, however its role in HNSCC is not well understood. Our lab has shown that amphiregulin (AREG), a ligand of the EGF receptor upregulates ANGPTL4 downstream. To test our hypothesis that ANGPTL4 plays a role in oral cancer cell migration, we used different oral cancer cell lines and treated with different concentrations of amphiregulin or other external stimuli, such as EGF or hypoxia, at specific concentrations to determine the expression of ANGPTL4, by western blot. The negative control measured ANGPTL4 without any condition and the positive control is EGF treatment. Using scratch assays, the effect of amphiregulin in oral cancer cell migration (and invasion) was measured. We found that as the concentration of AREG increased the expression of ANGPTL4 increased. For the scratch assays, AREG induced oral cancer cell migration. These results support that ANGPTL4 plays an important role in inducing cancer cell migration and invasion. Future directions would be to understand the exact role of ANGPTL4 in HNSCC development and metastasis, and its downstream signaling pathways associated with cell migration and proliferation.

Alexia Smith
Stevenson University
Mentor: Dr. Charles Hong

Impact of G Protein-Coupled Receptor 68 (GPR68) Inhibition on Glioblastoma Survival

Glioblastomas are aggressive, malignant cancer tumors found in the brain. Every year, more than 10,000 people in the United States will be diagnosed with glioblastoma. The average survival time is 12-18 months. Only 5% of patients survive past 5 years. G protein-coupled receptor 68 (GPR68) is a protein that is highly upregulated in cancer cells. GPR68 is important to a cancer cell's growth and metastasis. In this study, survival of a glioblastoma cell line (U87) was observed after inhibition of GPR68. We hypothesized that inhibition of GPR68 would encourage glioblastoma cells to undergo apoptosis. Two different methods were employed to observe the survival of U87 cells. The first method was accomplished by using varying concentrations of the inhibitor ogremorphin (OGM) and its analogs of GPR68 applied to the cell line in a structural activity relationship study (SARS) drug assay. The half minimal inhibitory concentration (IC₅₀) was then calculated for each drug. Additionally, knockdowns were performed by using small interfering RNA (siRNA) to transfect the cells and degrade the mRNA sequence coding for GPR68. After the knockdown, the cells underwent RNA extraction and quantitative PCR amplification (qPCR). The survivability of glioblastoma cells decreased at higher concentrations of OGM and its analogs. The standard maximum for an IC₅₀ is below 10 μ M. Several analogs had a calculated IC₅₀ and 95% confidence level under 10 μ M. Additionally, the siRNA knockdown effected overall survivability in comparison to the control. Due to glioblastoma's resistance to most commercial drugs currently on the market, a new approach of inhibiting GPR68 could potentially give more insight of how to treat these tumors.

Tobias Liska
Assumption College
Mentor: Dr. Eli Bar

MBNL1 Mediated Alternative Splicing of Genes Associated with Nervous System Development in Glioblastoma Multiforme

Glioblastoma multiforme (GBM) is an extremely aggressive grade IV astrocytoma with a dismal prognosis. Previous studies have shown that the activity of Muscleblind-like-protein 1 (MBNL1), a regulator of the alternative splicing of pre-mRNA into mature-mRNA, plays a major role in the progression of GBM; overexpression of MBNL1 inhibited proliferation and tumor growth *in vivo*, and GBM cells were shown to drive MBNL1 out of the nucleus. Alternative splicing is the process by which RNA is differentially spliced following transcription, allowing for a single gene to encode multiple isoforms of the same protein. This process occurs in around 80-90% of all genes, and therefore could be a target in studying GBM. In this study, we aimed to identify genes that were alternatively spliced by means of MBNL1. Analysis of multiomic data was performed to identify RNAs that were both bound to by MBNL1 and alternatively spliced when MBNL1 was overexpressed. The gene ontology analysis showed enrichment of genes associated with nervous system development. Alternative splicing patterns were assessed through PCR of complementary DNA (cDNA) derived from mRNA isolated from different GBM cell lines: parental, MBNL1 knockouts, MBNL1 overexpression, and cells cultured in hypoxia, a condition previously shown to inhibit MBNL1 splicing activity. Results revealed multiple genes that were confirmed to be alternatively spliced mediated by MBNL1. Furthermore, the analysis of the specific splicing patterns suggested that MBNL1 both promotes the inclusion and exclusion of certain exons that could play a vital role in determining the function of the protein.

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

Impact of RPTOR mutations on axon projection, correlation to brain cancer progression

The formation of the brain's intricate circuitry is driven by the extension of axons from neurons to their specific targets in different regions, forming connections throughout the brain. The growth cone of the axon is the director of this movement, and is guided to its proper target through interaction with axon guidance cues, as well as by the gene expression of the growth cone itself. Here we investigate the idea that genes prompting the extension and movement of axons may also play a similar role in encouraging the growth and spread of brain cancer, which is projected to claim the lives of 18,600 Americans this year alone. We used the cancer genomics database cBioPortal to investigate how survival rates of brain cancer patients were affected by the presence of mutations in genes that are enriched in the growth cone, one such gene being RPTOR. It was found that the median survival of patients with mutated forms of the RPTOR gene was nearly 50 months longer than that of patients with the wild-type gene, leading to the hypothesis that the disruption of genes implicated in growth cone movement would lead to decreased cancer growth and progression, as well as hindered axon projection. Seven of the mutations documented on cBioPortal were selected, and will be introduced using prime editing, initially *in vitro* to verify knockout efficiency before being introduced *in vivo* in mice through in utero electroporation. The brains of edited pups will be harvested and stained to visualize the axon projections formed by edited versus unedited neurons. We expect to see that the projection of axons expressing edited forms of RPTOR will be hindered, resulting in off-target or incomplete connections, and suggesting a correlation between genes implicated in growth cone movement and the progression of brain cancer.

Jennifer Yan

Duke University

Mentors: Graeme F. Woodworth, Anthony J. Kim, and Jeffery A. Winkles

Purification and Analysis of an Fn14 Monoclonal Antibody and its Antigen Binding Fab Fragment or Targeted Nanoparticle Cancer Therapy

Glioblastoma (GBM) is an aggressive form of brain cancer, with a median survival of 12-15 months from diagnosis. GBM cells show high invasion into the surrounding healthy brain tissue, preventing complete surgical resection and driving tumor recurrence. Furthermore, recurrent tumors are largely resistant to current therapies; thus, new drug delivery approaches and therapeutic agents are needed to improve patient outcomes. Our lab is developing cancer targeted nanotherapeutic (NP) platform containing a monoclonal antibody (mAb), ITEM-4, specific to the protein Fibroblast growth factor-inducible 14 (Fn14). Fn14 is a cell surface receptor for the tumor necrosis factor (TNF) family member TNF-like weak inducer of apoptosis (TWEAK), and has higher expression in GBM tumors compared to normal brain, making it a promising cell surface portal for NP drug delivery. The objective of this project was to purify ITEM-4 from hybridoma cell cultures and analyze surface conjugation of full length or fragmented mAbs onto NP surfaces. To this end, we attempted to purify hybridoma-secreted ITEM-4 by optimizing hybridoma culture conditions and isolating ITEM-4 from hybridoma conditioned media, using affinity-based protein purification techniques. Concurrently, a control purified ITEM-4 was fragmented using a Fab fragmentation kit. Subsequently, these mAbs were characterized for purity via SDS-PAGE, and Fn14 binding strength was assessed using Surface Plasmon Resonance (SPR) assays. The successfully purified ITEM-4 mAbs were thiol-modified and bio-conjugated onto the paclitaxel-loaded polymeric PLGA-PEG NPs surface. These assembled NPs were further characterized using dynamic light scattering techniques and SPR assays. Future studies will continue optimization of ITEM-4 mAb purification from hybridomas and assess the therapeutic value of ITEM-4 conjugated NPs in pre-clinical models to elucidate the clinical translation potential of Fn14 targeted nanoparticles for GBM therapy.

Samantha Rea

Stevenson University

Mentor: Dr. Ryan Pearson

Induction of CD8⁺ T cell-mediated cancer cell lysis via antigen-polymer conjugated nanoparticles

T lymphocyte focused immunotherapies show promising advances for cancer treatment, however complications which limit their clinical application include insufficient T cell expansion, inability to modulate hostile tumor microenvironments, difficulty infiltrating solid tumors, and toxicities. Due to their customizable nature, nanoparticles may potentially be designed to promote T cell expansion, overcome hostile tumor microenvironments, and minimize toxicities through sustained therapeutic release. In this study, we investigated the ability of antigen-conjugated, biodegradable, poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) to promote an anti-tumor response *in vitro*. PLGA nanoparticles conjugated with ovalbumin (OVA) (PLGA-OVA) were synthesized through a single emulsion technique and characterized via dynamic light scattering (DLS) and bicinchoninic acid (BCA) assay. To evaluate the ability of the NPs to induce cytotoxicity in cancer cells, bone marrow-derived dendritic cells (BMDCs) were treated with PLGA-OVA and co-cultured with isolated CD8⁺ T cells from OT-I mice, which are designed to recognize ovalbumin. The CD8⁺ T cells were subsequently isolated and co-cultured with an OVA-expressing murine melanoma cell line (E.G7-OVA) to induce lysis of the cancer cells. An LDH assay revealed that cytotoxicity was induced by the PLGA-OVA treatment compared to the low LDH control, thus suggesting that PLGA-OVA activates cell lysis via CD8⁺ T cell activation. Antigen-specific NPs offer a potential for a targeted immune-enhancing immunotherapy and drug delivery tool.

Kristen Zaranski
University of Maryland College Park
Mentor: Dr. Rena Lapidus

The effect of Doxycycline and Celecoxib on the growth and metastasis of HT-1080 fibrosarcoma and U-2OS osteosarcoma cell lines

Sarcoma is a type of cancer that arises from connective tissue, including bone, cartilage, fat, or blood vessels, with many cases being diagnosed in children. Surgery, chemotherapy, and radiation, common treatment modalities for this disease, are often unable to stop the growth of these aggressive tumors; therefore, new therapeutics are necessary. The present study examines three FDA approved drugs, Doxycycline, Celecoxib, and Minoxidil Sulfate, as well as the drug Meta-Tyrosine, and their effect on two sarcoma cell lines: HT-1080, a fibrosarcoma, and U-2OS, an osteosarcoma. By repurposing therapeutics, clinical trials can proceed more quickly. We first determined the IC₅₀ of these four agents in the two cell lines. Based on this data, we pursued Doxycycline and Celecoxib for continued studies. Migration and invasion assays, 'in vitro' metastasis models, showed a dose-dependent inhibition of cell movement across a membrane in the presence of Doxycycline and Celecoxib individually. Live-dead and annexin V assays measured cell viability and apoptosis respectively after these drugs were exposed to the two cell lines. Of the four agents tested, Celecoxib appears to be the most effective therapeutic treatment against the two sarcoma cell lines. Additionally, we evaluated the effects of Doxycycline and Celecoxib in combination on HT1080 in an in vivo mouse model. By pretreating the mice with the two agents before injecting HT-1080 cells, we were able to test if the tumors would attach and grow in the presence of Doxycycline and Celecoxib. Although the experimental group receiving the treatment grew tumors, this group grew tumors at a slower rate than the control group suggesting that the treatment slows the growth of HT-1080. It would be beneficial to continue studying cellular and molecular actions of Celecoxib on sarcoma cells in vitro and the therapeutic efficacy of combination with other drugs for tumor growth and metastasis in vivo for new clinical applications.

Jameila Barrett and Demi Morris
Stevenson University (JB)
The University of Maryland College Park and ACS-Diversity in Cancer Research Intern (DM)
Mentor: Dr. Paul Shapiro

Investigating Vulnerabilities of Mitogen Activated Protein Kinases in Diffuse Large B-cell Lymphoma

Lymphoma is a form of blood cancer that is caused by unregulated lymphocyte proliferation. The two main types of lymphoma include Hodgkin's lymphoma and non-Hodgkin's lymphoma. There are multiple categories of non-Hodgkin's lymphoma, including diffuse large B-cell lymphoma (DLBCL). DLBCL is an aggressive form of lymphoma that has two subtypes, germinal center B-cell (GCB) and activated B-cell (ABC). The ABC subtype has particularly low survival rates, meaning the creation of new and more effective therapeutic treatments for this disease is imperative. It is hypothesized that differences in MAPK signaling in the subtypes could be exploited to develop new therapies. Furthermore, what are the differences in MAPK signaling between the ABC and GCB subtypes? To complete this study, cellular proteins from HBL-1 (ABC subtype) and Toledo (GCB subtype) were separated by SDS-polyacrylamide gel, transferred to a PVDF membrane, and analyzed via immunoblotting. This allowed for detection of MAPK activity. Based on this we tested p38 and ERK inhibitors for effects on viability of Toledo and HBL-1 cells, respectively. Cell viability was then measured with CellTiter-Blue to observe the impact on the cells. Results from this study indicated that p38 was highly expressed in the Toledo cell line, whereas ERK 1/2 was highly expressed in the HBL-1 cell line. In addition, the SF-3-030 was a potent inhibitor of both subtypes. Further testing showed that SF-7-028 had the greatest impact on the HBL-1 cell line. The data from this study suggests that the use of these function selective inhibitors could potentially be applied to therapeutic treatments of ABC and GCB lymphoma.

Alyssa Low
Texas A&M University
Mentor: Dr. Gerald Wilson

A Non-Canonical Mechanism for the Antiproliferative Activity of Tristetraprolin

Breast cancer is the most prevalently diagnosed cancer and a leading cause of cancer deaths. Tristetraprolin (TTP) is a candidate tumor suppressor protein whose decreased expression correlates with aggressive cancer phenotypes and poor patient outcomes. To identify mechanisms connecting TTP to decreased tumor aggressiveness, TTP-expressing and control cell lines were constructed in the aggressive, triple-negative breast cancer cell model MDA-MB-231. Expression of TTP slowed cell proliferation and caused modest accumulation of cells in the S and G2 phases of the cell cycle. Western blots revealed no significant TTP-dependent changes in the expression of key cell cycle regulators aside from a marginal decrease in cyclin D1. TTP's canonical function is to accelerate mRNA decay through binding AU-rich elements, with many cognate mRNAs encoding factors associated with cancerous phenotypes. However, actinomycin time course assays revealed no significant destabilization of known TTP target mRNAs. Since ERK and p38^{MAP} kinases are constitutively active in MDA-MB-231 cells and can inhibit TTP's mRNA-destabilizing function, turnover of known mRNA targets was also measured in the presence of MAP kinase inhibitors. However, inhibiting these kinases did not restore rapid mRNA decay. Retention of the tumor-suppressive activity of TTP independent of its ability to degrade mRNA suggests that the tumor-suppressive activity of TTP employs non-canonical mechanisms. To elucidate candidate mechanisms, RNA-Seq analysis was performed to identify genes differentially expressed between TTP-expressing and control cell lines, which were then triaged based on impacts on patient survival and known roles in regulating cell proliferation. Candidate factors are currently being validated by qRT-PCR and Western blot, after which gain and loss of function studies will interrogate if these genes do indeed serve a role in the antiproliferative activity of TTP. A more comprehensive understanding of the antiproliferative mechanisms of TTP will be key in defining its diagnostic and therapeutic value.

Sophie Liu
Johns Hopkins University
Mentor: Dr. Michal Zalzman

The effect of ZSCAN4 on histone modifications and cell stemness

In recent years, cancer survival rates have increased from novel therapies. Despite these advances, tumor resistance to radiation and chemotherapy and risk of metastasis is still prevalent among patients. Cancer stem cells (CSCs) and mesenchymal stem cells (MSCs) share their ability to self-renew and proliferate. Alongside, CSCs can differentiate into heterogeneous cancerous cells, regrow tumor masses, and contribute to drug resistance. Subsequent work highlights common phenotypic markers and signaling pathways conserved in both MSCs and CSCs such as Hedgehog, TGF- β , Notch, and Wnt. In conjunction with prevalent core pluripotency factors (OCT3/4, NANOG, and SOX2), stem cells facilitate various histone modifications, transcriptional reprogramming, and chromatin reconfiguration. Our lab previously reported that endogenous ZSCAN4 increases chromatin activation, which corresponds with decreased DNA methylation and increased histone acetylation. Since multipotent stem cells (MSCs) are strongly suggested to harbor stem-like cell populations similar to CSCs, we hypothesized an induction of ZSCAN4 in MSCs would increase epigenetic and “stemness” profiles. MSC cells are described to have more open chromatin signatures than non-stem cells. To study the effect of ZSCAN4, we generated Doxycycline (Dox) inducible ZSCAN4 MSC cells. By adding doxycycline to our MSCs, we expected to see increased ZSCAN4 expression, yielding a phenotype of increased active histone marks, upregulation of pluripotency genes, and transcriptional changes. To test this hypothesis, Dox-inducible tonsil-derived human MSC lines were treated with doxycycline for 48 hours. Following treatment, cells were harvested or fixed for analyses. To validate the predicted increase in stemness signatures following dox-treatment, novel protein and gene targets were observed through western-blots, immunostaining, and RT-PCR. We show that ZSCAN4 induction causes clustering of heterochromatin around the nucleoli, coinciding with increased histone acetylation markers and upregulation in pluripotency gene expression. Understanding the hallmarks of ZSCAN4 in stem cells with its transient effects on transcriptional activity may elucidate stem cell and cancer stem cell profiles, leading to targeted agents in the future cancer therapeutics.

Sara Jain
University of Maryland, College Park
Mentor: Dr. Michal Zalzman

The effect of iCRISPR/Cas9 knockout of ZSCAN4 on cancer stem cell factors

Cancer stem cells (CSCs) are a rare subpopulation of cancer cells that reserve the ability to self-renew, differentiate, metastasize, and form tumors. Current cancer treatments efficiently target the bulk of the cancer cells in tumors. However, this small population of cancer stem cells use specific mechanisms which render them resistant to conventional radiation and chemotherapy treatments. We have recently shown that the early-embryonic stage gene ZSCAN4 plays a crucial role in maintaining the CSC phenotype. We demonstrated that ZSCAN4 leads to increased histone acetylation, which opens the chromatin and thereby leads to increased expression of stemness genes. To study the effect of ZSCAN4 knockout, we generated Doxycycline (Dox) inducible CRISPR/Cas9 U2OS cells. As controls, we used wild type (WT) and empty vector iCas9 without sgRNA. Cells were tested to study the role of ZSCAN4 in histone acetylation and pluripotency gene expression. We predicted that the iCas9 edited cells would display lower levels of pluripotency markers than both the iCas9 empty cells and the wild type U2OS cells. RT-qPCR and immunostaining were both used to show, quantitatively and qualitatively, the difference in expression of pluripotency markers. As expected, our results indicate that ZSCAN4 knockout leads to downregulation of pluripotency genes. Defining possible pluripotency markers and methods by which ZSCAN4 may be involved with cancer stemness may provide novel targets for drug design in cancer therapies.

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Mentor: Dr. Nykia Walker

Early Predictive Marker of Breast Cancer Metastasis

Pathological and clinical assessment of predictive markers for breast cancer (BC) are instrumental to help in decision making and clinical treatment recommendations. The Nottingham histologic score (NHS), is a histologic grading system, analyzes a plethora of predictive histomorphology features under three main categories: tubule formation, nuclear pleomorphism and mitotic activity. The combination of the NHS categories can predict BC specific survival, disease free survival and tumor aggressiveness. However, interobserver variation of histologic assessment is an increasing area of concern and current software tools that mitigate this variation are expensive. Therefore, there is a need for more cost effective alternatives to analyzing histomorphology. In my project, I used the Nottingham Histologic Score (NHS) to analyze histomorphological features of Triple Negative Breast Cancer (TNBC) in xenograft mice models. I used 3 out of the 5 NHS categories to analyze samples from 4 different mice exposed to 4 different treatment groups to examine morphology changes in distant tissue. I used ImageJ and Adobe to quantify the following histomorphology features: nuclear to cytoplasm ratio (N/C), nuclear pleomorphism, and prominence of nucleoli in mice with and without BC. Both histomorphological features were used to analyze greater than 1000 cells among different groups and were imaged using the Leica SP5 confocal microscope at 63x magnification. N/C is defined as the cross sectional area of the nucleus divided by that of the cytoplasm. Nuclear pleomorphism is commonly used to categorize and score BC tissue. Prominent nucleoli are indicated by dark circular figures in cells. Increased N/C was observed in the BC group compared to mice without BC. Nuclear pleomorphic features and prominent nucleoli were observed with significant changes in BC tissue. Our findings have implications as an early predictor of disease in non-cancerous tissue as well as being a good tool to implement artificial intelligence models.

Ally Jerrett
Loyola University Maryland
Mentor: Dr. Stuart S. Martin

Discovery of ROS-induced Tubulin Detyrosination and Acetylation in Breast Cells

The mechanism by which cancer cells sense and respond to their mechanical environment contributes to metastasis. Microtubules are essential for many cellular functions and can be modified through the addition or removal of amino acids and side groups. One such modification is the removal of the C-terminal tyrosine from alpha-tubulin, termed detyrosinated tubulin (deTyr-Tub). Microtubules are sensitive to mechanical stimulation and the subsequent generation of reactive oxygen species (ROS) is essential for mediating the mechanotransduction pathway. In normal breast epithelial cells and their oncogenic counterparts, ROS induces an increase in deTyr-Tub. DeTyr-Tub is associated with a poorer prognosis in breast cancer patients and is found at the leading edge of invasive tumor fronts. This project serves to understand the novel mechanism by which ROS induces deTyr-Tub. When directly treated with H₂O₂, MCF10A cells increase deTyr-Tub at concentrations as low as 500μM, with a peak response resulting around 10mM H₂O₂ at treatment times ranging from 30 minutes to 4 hours. MCF10As with a constitutively active KRAS mutation had a greater increase in deTyr-Tub at lower concentrations compared to normal MCF10As. Our findings suggest that ROS induced deTyr-Tub appears to be independent of extracellular calcium intake as well as intracellular calcium stores. Additionally, ROS increases other tubulin PTMs in MCF10As including alpha tubulin acetylation at lysine 40. Our results suggest that ROS is not directly modifying tubulin or disrupting overall filament organization, but rather is stimulating an enzyme dependent mechanism. Since ROS is often elevated in the tumor microenvironment and essential to the mechanotransduction pathway, understanding how ROS affects microtubules can offer insights on how tumors progress and cancer cells metastasize. Future studies should continue to explore how ROS facilitates detyrosination of microtubules and affects overall microtubule structure as well as tumor cell phenotypes, like migration and invasion.

Lena King
University of Notre Dame
Mentor: Dr. Cristiana Cairo

Factors Associated with Cytotoxic Potential in Neonatal Vd2 Cells

Vg9Vd2 (Vd2) T cells in healthy adult individuals are potent cytotoxic effectors. They are an appealing candidate for cancer immunotherapy due to their broad, MHC-independent recognition of solid and hematologic tumors. However, tumor-infiltrating Vd2 cells often have poor cytotoxic function, limiting their therapeutic potential. Acquisition of robust cytotoxic function mostly occurs in the first two weeks of life, but the factors promoting this differentiation process have not been characterized. Details about the differentiation mechanisms may help reprogram Vd2 cells into functional cytotoxic effectors. We are using a two-week culture of cord blood Vd2 cells to mimic cytotoxic effector differentiation in early life and study determinants of cytotoxic function. We analyzed neonatal Vd2 cytotoxic potential *ex vivo* and after expansion, monitoring the expression of perforin and CD107a in relation to PD-1 and CD56. CD56 was previously associated with adult Vd2 cell cytotoxicity, PD-1 appears to modulate neonatal Vd2 cell cytotoxicity based on our results. After expansion, we observed that the PD-1+ CD56- subset contained a low percentage of perforin+ and CD107a+ cells (mean: 20% and 25%, respectively), while the PD-1- CD56+ subset contained a high frequency of cytotoxic effectors (mean: 70% and 65%, respectively). To further characterize the functional differences between PD-1+ CD56- and PD-1- CD56+ cells, these subsets were sorted for bulk RNAseq. One of the differentially regulated genes, the transcription factor IRF8, may be involved in the differentiation of cytotoxic Vd2 effectors. RT-PCR analysis confirmed that neonatal PD-1- CD56+ cells express about 5-fold higher IRF8 levels than the PD-1+ CD56- cells, consistent with our hypothesis. These results warrant further RNA interference or transduction studies to test what happens to Vd2 cytotoxic potential when IRF8 levels are forcibly decreased or increased in primary adult or neonatal cells. Our results may inform strategies to maximize Vd2 cell therapeutic potential for cancer immunotherapy.