The 2018 Summer Research Symposium of the Nathan Schnaper Intern Program in Translational Cancer Research

25 July 2018
Southern Management Corporation Campus Center

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

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NSIP
Nathan Schnaper Intern Program
in Translational Cancer Research

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

2018 NSIP Research Symposium

Wednesday, July 25, 2018
8:00 am to 5:20 pm
SMC Campus Center room 351

	Speaker	Mentor
8:00 to 8:30 am	**Breakfast**	
8:30 to 8:40 am	**Bret A. Hassel, PhD** *Director's Welcome*	
8:40 to 9:00 am	**Karleigh Baldwin** *Investigation of the Interaction between the RGG Motif of hnRP A18 and Potential Drug Candidates*	David Weber
9:00 to 9:20 am	**Joshua Bell** *The Effects of Tristetraprolin on Cell Proliferation and NF-κB Signaling in Triple Negative Breast Cancer Cell Lines*	Gerald Wilson
9:20 to 9:40 am	**Malia Vester** *Evaluating acquired resistance to kinase inhibitors in melanoma cells*	Paul Shapiro
9:40 to 10:00 am	**Zachary Michel** *Utilizing lipid tethering technology allows for unique analysis of metastatic breast cancer cells*	Stuart Martin
10:00 to 10:20 am	**Alyssa Hubal** *The Maintenance of the Cancer Stem Cell Phenotype*	Michal Zalzman
10:20 to 10:40 am	**Break**	
10:40 to 11:00 am	**Benjamin Merenbloom** *Amphiregulin upregulates ANGPTL4 to promote lymphangiogenesis in head and neck squamous cell carcinoma*	Silvia Montaner
11:00 to 11:20 am	**Morgan Taylor** *Identification of DNAJB1-PRKACA Fusion Transcript in One Patient with Fibrolamellar Carcinoma*	Scott Strome
11:20 to 11:40 am	**Emily Shukdinas** *Role of DACH1 in TF1 Cell Growth and Erythropoiesis*	Tami Kingsbury

11:40am to 12:00pm	**Claudia Atwood** *The Reprogramming of Tumor-Specific T-Cells using the IL-12p40 in IFNγ Pathway for Tumor Clearance*	Nevil Singh
12:00 to 12:20 pm	**Alicia Dent** *Characterization of Novel Mnk1 and Mnk2 Degrading Agents in Models of Triple Negative Breast Cancer (TNBC)*	Vincent Njar
12:20 to 1:20 pm	**Lunch**	
1:20 to 1:40 pm	**Aaron Pomerantz** *Exchanging GFP for LNGFR(CD271) in Fusion Protein Constructs for Magnetic Bead Isolation of Transduced T Cells*	Eduardo Davila
1:40 to 2:00 pm	**Reena Goswami** *PARP Inhibitors and DNA Methyltransferase Inhibitors as a Synergistic Drug Combination to Treat Cancer*	Feyruz Rassool
2:00 to 2:20 pm	**Arsene Noe** *The Effect of MCD-66 in Combination with Chemotherapy in Acute Myeloid Leukemia*	Rena Lapidus
2:20 to 2:40 pm	**Jacqueline Hwang** *Comparative cytotoxicity analysis of free drug, targeted, and non-targeted drug-loaded nanoparticles in MDR1-positive cancer cells*	Winkles/Kim/Woodworth
2:40 to 3:00 pm	**Sara Fioretti** *Analyzing effect of nanoparticle shape on immune cell biodistrubution in 4T1 model of breast cancer*	Gregory Szeto
3:00 to 3:20 pm	**Danielle Cannady** *Impact of the obscurin-PH domain in cell growth, motility and invasion*	Aikaterina Kontrogianni - Konstantopolous
3:20 to 3:40 pm	**Break**	
3:40 to 4:00 pm	**Carina Sclafani** *Targeting Apurinic Endonuclease 1 (APE1) Using Fragment Based Drug Design*	Alex Drohat
4:00 to 4:20 pm	**Richa Beher** *Concurrent Treatment of PARP I Inhibitor and TOP2 Inhibitor Increases DNA Damage and Apoptosis in Acute Myeloid Leukemia Cells with FLT3-ITD*	Maria Baer
4:20 to 4:40 pm	**Ryan O'Donnell** *CD27 mediated regulation of allogeneic T cell response*	Xuefang Cao

4:40 to 5:00 pm	**Olivia Konen** *Determination of the Consequences of Disrupting Interaction Between MYH Repair Enzyme and the Checkpoint Clamp*	A-Lien Lu-Chang
5:00 to 5:20 pm	**Lyra Morina** *Contribution of Starvation Factors to Autophagic Signaling and Degradation*	William Jackson

Abstracts

(in speaking order)

Karleigh Baldwin
Stevenson University
Mentor: Dr. David Weber

Investigation of the Interaction between the RGG Motif of hnRP A18 and Potential Drug Candidates

Heterogeneous nuclear ribonucleoprotein A18 (A18) is part of a family of RNA binding proteins, which interacts with mRNA transcripts under different cellular conditions. A18 recognizes a 51 nucleotide signature motif in the 3' Untranslated Region of pro-survival mRNAs, such as hypoxia inducible factor 1- α (HIF-1 α), during cytotoxic stress. In response to low levels of oxygen usually in the center of solid tumors, A18 aids in the stabilization and upregulation of pro-survival transcripts in colon, breast, prostate, and melanoma tumors for cellular survival. A18's structure contains an RRM motif (RNA Recognition motif) and an RGG motif (Arginine-glycine-glycine motif), both of which are required for optimum binding of A18 to the targeted mRNA transcripts. RRM motifs contain highly conserved residues important for mRNA binding. RGG motifs are normally involved in protein-protein as well as protein-nucleic acid interactions. This RGG motif of A18 is required for optimal RNA interactions, but how it impacts this interaction at the molecular level has yet to be determined. Small molecule candidates have been demonstrated to interact with the A18 RRM, but they have not been investigated for interaction with the RGG motif. The purpose of this study was to determine if the A18 RGG motif interacted with chosen drug candidates that have been demonstrated to interact with the RRM through use of nuclear magnetic resonance (NMR). Small molecules that interact with both the RRM and the RGG motif would provide increased ability to inhibit the interaction of A18 with pro-survival mRNA transcripts upregulated in cancerous tissue, so studies were initiated that examined small molecules binding to A18 and the results from these studies will be discussed.

Joshua Bell
Univ. of Maryland Baltimore County
Mentor: Dr. Gerald Wilson

The Effects of Tristetraprolin on Cell Proliferation and NF-κB Signaling in Triple Negative Breast Cancer Cell Lines

Tristetraprolin (TTP) is an RNA-binding protein that accelerates decay of mRNAs containing AU-rich elements. The expression of TTP is commonly suppressed in aggressive breast cancer cells, and low levels of TTP expression are associated with poor patient outcomes. These factors suggest that TTP may function as a tumor suppressor protein. To test whether TTP might impact tumor growth by limiting cell proliferation, trypan blue exclusion assays were performed to measure the growth rates of aggressive breast cancer cell lines MDA-MB-231 and MDA-MB-436. Both lines lack detectable endogenous TTP, so proliferation was compared between lines expressing FLAG-tagged TTP versus an empty vector control. Since previous data showed that TTP suppresses the transcription factor c-Myc, a central driver of glucose addiction in several cancer cell models, an additional experiment measured the effect of TTP on MDA-MB-231 proliferation under conditions of glucose deprivation (1 mM) vs. normal glucose conditions (10 mM). Finally, a published report suggested that TTP might inhibit tumor formation by MDA-MB-231 cells by suppressing NF-κB signaling, which is enhanced in many cancers and promotes gene regulatory pathways that promote cell proliferation and protect cells from apoptosis. We tested the hypothesis that TTP suppresses NF-κB activity in MDA-MB-231 cells by measuring the effect of TTP on expression of the NF-κB-responsive gene interleukin-8, as well on transcription from an NF-κB reporter plasmid.

Malia Vester
Coppin State University
Mentor: Dr. Paul Shapiro

Evaluating acquired resistance to kinase inhibitors in melanoma cells

Genetic mutations contribute to the development of many cancers. In melanoma one of these mutations is BRaf V600E that increases the growth and spread of cancer by turning on MEK 1 / 2 and ERK 1 / 2. The activation and deactivation of the Braf effector protein in the mitogen activated protein kinase pathway are key to monitoring the cell proliferation and prohibit cell survival in melanoma. Inhibitors to ensure successful deactivation if the BRaf effector protein were development however after several months of usage patients began to acquire resistance. To overcome this acquired resistance, MEK 1 / 2 and ERK 1 / 2 inhibitors where developed. In this study, IC50 of cells growing in the presence of BRaf, MEK 1 / 2, and ERK 1 / 2 inhibitors were identified in aim to evaluate cell proliferation through usage of crystal violet assay. The objective aim of this study is to establish drug resistant cells and extract data to evaluate the mechanism drug resistance.

Zachary Michel
State College of Florida
Mentor: Dr. Stuart Martin

Utilizing lipid tethering technology allows for unique analysis of metastatic breast cancer cells

In late stages of cancer, primary tumors become more likely to shed cells into the bloodstream which poses a risk for the development of metastatic disease. These circulating tumor cells (CTCs) have previously been shown to modulate survival and apoptotic pathways in order to persist in their detached state as well as promote reattachment to distal tissue by expressing tubulin-based microtentacles (McTNs). Until recently, analysis of CTC characteristics has been limited due to a lack of efficient *in vitro* and *in vivo* methods. Utilizing a microfluidic substrate coated with a polyelectrolyte multilayer (PEM), an additional layer of lipid may be deposited which is able to tether cells in place due to interactions with the phospholipid bilayer of the cell membrane. While tethered, cells have been observed to maintain free-floating characteristics such as a more rounded cell morphology in epithelial-like cells and McTN expression in tumorigenic and more aggressive cell lines, suggesting that tethered cells may behave as if there were free-floating. In order to test the ability of lipid tethering to mimic the free-floating conditions of CTCs, the MDA-MB-436 cell line was used in tethering experiments and subjected to multiple different wash conditions to assess the ability of the lipid to keep cells tethered despite physical agitation. There was greater than 90% cell retention on the lipid tethers following multiple washes of either formaldehyde-fixed or live cells. Additionally, cells permeabilized with Triton-X were also shown to have greater than 90% cell retention following fixation to the lipid layer whereas the vast majority of cells only exposed to PBS were washed away following permeabilization. These results display the reliability of lipid tethering to keep cells in place, allowing for observation over time. Future studies should continue to evaluate if lipid tethering confers a viable imitation of a free-floating environment; moreover, lipid tethering may eventually allow for validation studies of McTN dynamics and drug response, elucidating the development of metastasis.

Alyssa Hubal
Loyola University Maryland
Mentor: Dr. Michal Zalzman

The Maintenance of the Cancer Stem Cell Phenotype

Cancer stem cells represent a subset of cells within a tumor that have the ability to either self-renew into more cancer stem cells or differentiate into malignant cells that contribute to the bulk tumor. Current cancer drugs target and eradicate the rapidly proliferating cells. However, cancer stem cells remain dormant during treatment, contributing to therapeutic resistance leading to tumor reoccurrence. Recently, researchers discovered that embryonic factors are regulators of cancer stemness, making them therapeutic targets. Research from the Zalzman lab has shown that the embryonic factor, ZSCAN4, plays a role in supporting the cancer stem cell phenotype. However, the mechanism by which ZSCAN4 acts to maintain the stem cell phenotype is unknown. To determine the role of ZSCAN4 in cancer stem cells, we used tetracycline (tet) inducible expression system to study the effect of ZSCAN4 induction on cancer stem cell division. We cultured and passaged three different cell lines with the tet-system including breast (SKBR3) and head & neck cancer (Tu167) cell lines. Immunostaining for cancer stem cells markers and flow cytometry were then used to differentiate between cancer stem cell populations both before and after induction. An increase in the number of undifferentiated cancer stem cells present after induction may indicate that the embryonic factor maintains the stem cell phenotype. Overall, our study aims to define the role of ZSCAN4 in the maintenance of the undifferentiated state and survival of cancer stem cells which could implicate ZSCAN4 as a potential cancer therapeutic target.

Benjamin Merenbloom
Univ. of Maryland College Park
Mentor: Dr. Silvia Montaner

Amphiregulin upregulates ANGPTL4 to promote lymphangiogenesis in head and neck squamous cell carcinoma

Head and neck cancer represents the sixth most common cancer worldwide. More than 90 percent of head and neck cancers are squamous cell carcinomas (HNSCC) that arise in the oral cavity, the oropharynx, and the larynx. The presence of lymph node metastasis determines the prognosis and survival of affected individuals. Lymph node metastatic tumors occur in about 40 percent of patients with oral cancer, highlighting the urgency to identify new targets for the early prognosis and management of HNSCC. In our lab, we found that Angiopoietin-like 4 (ANGPTL4) is upregulated in preneoplastic and neoplastic oral squamous cell carcinoma cells and may serve as a molecular marker in HNSCC. Interestingly, epidermal growth factor receptor (EGFR) overexpression, a molecular hallmark in oral cancer, increases ANGPTL4 levels in HNSCCs. The aim of this project was to determine the EGFR-dependent extracellular signal that triggers the upregulation of ANGPTL4 in HNSCC progression. For this purpose, we used an oral preneoplastic cell line, dysplastic oral keratinocytes (DOKs), an oral cancer cell line (HN-6), and normal oral keratinocytes (NOKs) and studied the upregulation of ANGPTL4 by different EGFR ligands. We found that Amphiregulin (AREG), a growth factor that induces cell proliferation and differentiation upon binding to EGFR, upregulates ANGPTL4 in DOKs. Interestingly, ANGPTL4 led to the migration of lymphatic endothelial cells in vitro, a mechanism involved in the formation of new lymphatic vessels. Collectively, these results suggest that AREG induces the upregulation of ANGPTL4 and may help in the promotion of lymphangiogenesis in HNSCC. These results further propose that ANGPTL4 may be a molecular target for oral cancer dissemination.

Morgan Taylor
Univ. of Maryland Eastern Shore
Mentor: Dr. Scott Strome

Identification of DNAJB1-PRKACA Fusion Transcript in One Patient with Fibrolamellar Carcinoma

Fibrolamellar Carcinoma (FLC) is a distinct hepatocellular carcinoma that classically occurs in young individuals with no antecedent risk factors. Recently, the DNAJB1-PRKACA fusion protein was identified as the driver mutation in FLC. The aim of my project was to determine whether this fusion mutation was present in one patient with FLC. DNA and RNA was extracted from the tumor and normal tissue. Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was performed to detect DNAJB1-PRKACA fusion transcript. The results of our study confirmed the presence of the DNAJB1-PRKACA in tumor, but not normal tissue. Because this mutation has previously been shown to be present in all tumor cells, we hope that in the near future we can use it as a basis for vaccine development.

Emily Shukdinas
Loyola University Maryland
Mentor: Dr. Tami Kingsbury

Role of DACH1 in TF1 Cell Growth and Erythropoiesis

The PAX-SIX-EYA-DACH Network (PSEDN) is required for the development of multiple tissues, via regulation of cell proliferation, stem-progenitor cell self-renewal and differentiation. Altered expression levels of network members has been linked to the initiation and progression of various cancers. A *Drosophila melanogaster* second site non-complementation screen conducted revealed that PSEDN members are factors that genetically interact with the GATA-FOG network. The lab has studied the role of SIX and EYA to determine if regulation of the GATA-FOG network by the PSEDN is conserved from fly to humans. Using the TF1 erythroleukemia cell line, our lab is interested in investigating the role of DACH1 in leukemia and erythropoiesis. TF1 cells express the hematopoietic stem-progenitor cell marker CD34 and undergo erythroid differentiation in response to erythropoietin. This differentiation is monitored by increased surface expression of transferrin receptor (CD71) and glycophorin A (CD235a), reduced CD34 expression, and occurrence of the “GATA switch”, in which the transcription factor GATA2 is replaced by GATA1. To test the consequences of DACH1 overexpression, TF1 cells were transduced with DACH1 expressing GFP marked lentivirus. DACH1 transduced TF1 cells exhibited reduced cell growth in both long-term GFP competition assays and short-term trypan blue cell counting assays. Flow cytometric analysis of erythroid markers further demonstrated that lentiviral mediated DACH1 overexpression increased CD71 and CD235a and reduced expression of CD34 compared to control transduced cells. These observations, suggest DACH1 overexpression stimulates erythropoiesis. Protein analysis of DACH1 overexpression cells should confirm increased differentiation by increased levels of GATA 1 and decreased levels of GATA2. Using the canonical CRISPR/Cas9 system, we are generating DACH1 knockout cells to further assess the role of DACH1 on TF1 cell growth and erythropoietic capacity.

Claudia Atwood
Virginia Polytechnic Institute and State University
Mentor: Dr. Nevil Singh

The Reprogramming of Tumor-Specific T-Cells using the IL-12p40 in IFNγ Pathway for Tumor Clearance

Successful rejection of tumors by the immune system requires T cells that have been suitably programmed to attack and kill malignant cells. This programming requires the cytokine interleukin-12 (IL-12), made by phagocytic cells near the tumor site, which instructs T cells to activate the interferon gamma (IFNγ) pathway for tumor reduction. For this reason, IL-12 is considered a strong candidate for enhancing immunity to a variety of cancers. Indeed, early clinical trials administering IL-12 directly to cancer patients showed promise; however, side effects from systemic immune damage due to the induction of IFNγ were seen. An approach which maintains the strong antitumor effect of IL-12 but eliminates the systemic adversities would be an ideal immunotherapy. One strategy is to administer IL-12 in pieces, such that it is inert until it combines into fully functional cytokine near the tumor. IL-12 is amenable to such an approach because it is a heterodimer composed of two proteins, p35 and p40. Importantly, our lab has previously shown that these subunits can combine extracellularly to form IL-12. P35 is expected to be released by necrotic cells in the tumor. Therefore, if recombinant p40 is administered in high doses, then p35 released locally at the tumor site should combine with p40 and the resulting IL-12 should lead to activation of tumor-specific effector cells without harmful non-tumor-specific activity. This hypothesis was tested using p40-/-, p35-/- and B6 WT mice with the EG7 lymphoma model. First, recombinant p40 was generated in HEK293 cells and purified using affinity chromatography. EG7 tumor cells were injected s.c. and allowed to grow until a measurable tumor appeared. Mice were then treated with 10 μg of recombinant p40 i.p. and tumor growth was followed. In addition to measuring tumor development (+/- p40), T cell activation will be analyzed. Draining and non-draining inguinal lymph nodes, spleen and tumor will be collected from all mice. T cells in each sample will be isolated and evaluated for IFNγ production using ICS Flow Cytometry. Based on these experiments we expect to propose p40 as a potential new immunotherapy against tumor growth.

Alicia Dent
Andrews University
Mentor: Dr. Vincent Njar

Characterization of Novel Mnk1 and Mnk2 Degrading Agents in Models of Triple Negative Breast Cancer (TNBC)

In many cancers, overactivation of biological pathways contribute to cell growth, proliferation and increased cell viability. The mTORC and RAS pathways are two of these that are often significant in cases of TNBC. These two pathways converge at the eukaryotic translation initiation factor (eIF4E), one of several proteins that make up the eIF4F complex. This complex is significant as it signals translation of potentially oncogenic proteins that aid in the growth and development of TNBC. Previous research has shown that the 4 compounds (novel retinamides) involved in my study are effective at degrading MAPK interacting kinases (Mnk1/2). These kinases are an integral part of the function of the eIF4F complex. They phosphorylate eIF4E, allowing the entire complex to be activated and initiate protein translation. My project seeks to confirm the hypothesis that oncogenic proteins translated due to the activation of this initiation complex will be down regulated upon degradation of Mnk1/2.

Aaron Pomerantz
University of Delaware
Mentor: Dr. Eduardo Davila

Exchanging GFP for LNGFR(CD271) in Fusion Protein Constructs for Magnetic Bead Isolation of Transduced T Cells

While T cell immunotherapy has shown promising results in the treatment of several blood cancers, its efficacy in treating solid tumors remains low. Solid malignancies exhibit an immunosuppressive tumor microenvironment (TME) that limits the ability of cytotoxic T lymphocytes (CTLs) from mounting sustainable antitumor responses. Because of such conditions, T cells exhibit exhausted phenotypes characterized by decreased antigen-specific activation, decreased proliferative capability and effector function, metabolic dysfunction, and an inability to generate long-lived memory T cells. Adoptive cell transfer (ACT) therapies are an attractive approach to overcome the immunosuppressive TME, as CTLs are typically modified *ex vivo* to enhance their *in vivo* antitumor functions. For example, our lab has shown that toll-like receptor (TLR) signaling activation in CTLs enhances antitumor activity. We recently devised a strategy to limit TLR stimulation of tumor-reactive CTLs through the expression of a CD8α:MyD88 fusion protein. CTLs expressing this protein harnessed MyD88 costimulation in a TLR-independent, yet tumor antigen-dependent manner, which resulted in enhanced antitumor activity *in vitro* and *in vivo*. To further this therapeutic platform, we've generated three novel fusion proteins that link MyD88 to subunits of the CD3 and CD4 co-receptors, and are investigating the impact of MyD88 signaling in T cells. We hypothesized that engineering T cells to express CD3εMyD88, CD3ζMyD88, or CD4MyD88 fusion proteins will result in a lower threshold for CTL activation and therefore enhanced antitumor activity. The objective of this experiment is to develop a rapid protocol for the analysis of MyD88 signaling activation in MyD88-stimulated versus non-stimulated T cells. The expression of Green Fluorescent Protein (GFP) downstream of the fusion proteins allows for separation of transduced CTLs from non-transduced cells, but its use has several therapeutic limitations in humans. The goal of this project, therefore, was to exchange GFP for LNGFR (CD271), a gene which can be used for magnetic bead separation. This process for separation is rapid, serving as a more favorable alternative to fluorescence-activated cell sorting. Here, we report the successful cloning of LNGFR downstream of two of our fusion proteins. CD3εMYD88-eGFP, CD3ζMYD88-eGFP, CD4MYD88-eGFP, and pUC-LNGFR plasmids were grown in bacterial cells, followed by DNA isolation via cell lysis and plasmid purification. Restriction enzyme digestion was used to excise GFP and LNGFR from their respective backbones. Isolated CD3εMYD88-, CD3ζMYD88-, CD4MYD88- backbones were then ligated with the digested LNGFR. Upon successful ligation, plasmid DNA was used to transform bacteria for plasmid synthesis. Diagnostic digests and DNA sequencing confirmed successful excision of GFP and ligation of LNGFR with the CD3εMYD88- and CD4MYD88- backbones.

Reena Goswami
Georgetown University
Mentor: Dr. Feyruz Rassool

PARP Inhibitors and DNA Methyltransferase Inhibitors as a Synergistic Drug Combination to Treat Cancer

Poly ADP-Ribose Polymerase inhibitors (PARPis) block the action of PARP1, which plays a key role in DNA single-strand break (SSB) repair, by trapping the enzyme at DNA damage sites. Clinically, PARPis have been investigated to sensitize cancers to radiation or to treat those with defects in homologous recombination (HR), such as BRCA deficiency. However, to expand use of PARPis in BRCA wild-type cancers and to treat emerging resistance to these inhibitors, we investigate a potential therapeutic combination of PARPis and DNA methyltransferase inhibitors (DNMTis) that disrupt gene expression. Previous studies by our lab indicate a synergistic model in which PARPis and DNMTis interact at sites of DNA damage, increasing PARP and DNMT trapping onto DNA. This complex blocks access of repair factors, alters gene expression patterns to reduce DSB repair, and causes accumulation of SSBs that become cytotoxic DSBs when encountered by the replication fork. To elucidate the mechanism of PARPis and DNMTis, we investigated if there is co-localization of the two enzymes that enhances trapping at DNA damage sites. To achieve this, we optimized the Proximity Ligation Assay (PLA), an immunoassay in which co-localized proteins are targeted by probes that engage in rolling circle DNA synthesis to produce fluorescent foci. In H460 non-small cell lung cancer cells, pre-treatment with a PARPi-DNMTi combination produced significantly more PARP1+DNMT1 foci, as well as more PARP trapping onto undamaged (PARP1+H2AX) and damaged (PARP1+yH2AX) DNA than vehicle or single agent treatment. Exposure to ionizing radiation enhanced these effects, suggesting a therapeutic combination with radiation. Similarly, MOLM14 acute myeloid leukemia cells pretreated with PARPi-DNMTi produced more co-localization of PARP1+yH2AX than vehicle or single agent treatment. These results support our synergistic model of PARPi-DNMTi enhanced trapping at DNA damage sites, and this protocol can be translated for use in correlative studies in our ongoing AML clinical trials.

Arsene Noe
Morgan State University
Mentor: Dr. Rena Lapidus

The Effect of MCD-66 in Combination with Chemotherapy in Acute Myeloid Leukemia

In 2018, 19,520 new cases of Acute Myeloid Leukemia (AML) will be diagnosed. AML is a type of cancer in which there are too many immature blood forming cells in the bone marrow and peripheral blood. The five year survival for patients older than 65 is 27.4%. AML is a large unmet medical need. The FLT-3 (Fms-like Tyrosine kinase 3) Internal Tandem (ITD) mutation is a poor prognostic indicator and occurs in approximately 30% of AML patients (Levis, 2013). Both Cytarabine (AraC) and Daunorubicin "7+3" induction are standard of care treatments in AML patients who can tolerate chemotherapy. Very few agents have been approved for AML in the last 40 years. We have been studying a binapthoquinone called MCD-66. In culture, this agent has a 0.3 uM IC50 against AML cell lines and also induces apoptosis, DNA damage and reactive oxygen species. In vitro and in vivo studies of MCD-66 as a single agent did not work well which is why combination therapy of this agent with chemotherapy drugs such as Cytarabine (AraC) and Gilteritinib (a FLT-3 ITD inhibitor) were tested in AML cell lines. This research evaluated the effects of MCD-66 in combination with AraC and Gilteritinib in AML cell lines, MOLM-14. We hypothesized that a sublethal dose of MCD-66 will potentiate the anti-proliferative activity of AraC and Gilteritinib in combination. Cells were exposed to a dose response of MCD-66, AraC and Gilteritinib for 72 hours to generate IC50 values. Two concentrations of MCD-66, an IC20 (0.15µM) and ~IC10 (0.1µM) were used in combination with a dose response of AraC and Gilteritinib. The results were read using MTT dye which measures mitochondrial function, a surrogate for cellular proliferation. Based on the observations, MCD-66 did not potentiate the effects of AraC or Gilteritinib. These results confirmed that MCD-66 at these concentrations do not increase the anti-proliferative activity of those two drugs. Future directions include the assessment of MCD-66 alone and in combination in non FLT3 mutated driven cancers with AraC and Daunorubicin.

Jacqueline Hwang
Dickinson College
Mentors: Drs. Winkles, Kim, and Woodworth

Comparative cytotoxicity analysis of free drug, targeted, and non-targeted drug-loaded nanoparticles in MDR1-positive cancer cells

One of the greatest challenges in modern cancer therapy is overcoming drug resistance mechanisms employed by cancer cells. It is well recognized that many cancer cells express ATP-binding cassette (ABC) transporters, for example, multidrug resistance (MDR) protein 1 (MDR1, also known as P-glycoprotein), which confer resistance to cytotoxic and targeted therapies by "pumping" drugs out of the cell. Previous research has suggested that nanoparticle-mediated drug delivery may overcome MDR by both enhancing drug uptake and reducing drug efflux via ABC transporters. Fn14 is a cell surface receptor present on most cancer cells, and we hypothesized that drug delivery via Fn14-targeted nanoparticles may overcome MDR mechanisms since the drug is delivered into the cytoplasmic space via receptor-mediated endocytosis, not passive diffusion. To test this, we obtained human Hey-8 ovarian cancer cells that expressed high levels of both Fn14 and MDR1 and cytotoxicity assays were conducted to compare cell viability after four different treatments: 1) free drug (paclitaxel (PTX)), 2) Abraxane, an FDA-approved PTX-albumin nanoparticle, 3) Fn14-targeted, PTX-loaded nanoparticles, and 4) non-targeted, PTX-loaded nanoparticles. We found that the three nanoparticle formulations had increased cytotoxic activity compared to the free drug when added to the MDR1-overexpressing cells. However, there were no significant differences in viability within the respective nanoparticle treatments. Future studies will repeat these experiments using additional drug resistant cancer cell types and optimize nanoformulations for maximal drug sensitivity.

Sara Fioretti
University of Mary Washington
Mentor: Dr. Gregory Szeto

Analyzing effect of nanoparticle shape on immune cell biodistrubution in 4T1 model of breast cancer

Cancer is known to hijack and suppress certain immune cells to grow and avoid destruction by the immune system. Previous studies showed that eradication of one suppressive cell type, myeloid derived suppressor cells (MDSCs), enhanced the efficacy of immunotherapy in the 4T1 murine model of breast cancer. Methods for eradicating MDSCs use nonspecific, potentially toxic agents, however, nanoparticles provide unique therapeutic opportunities by carrying high doses of drugs to specific cells using a targeting molecule such as an antibody. To date, active targeting using a cell specific molecule has been the predominate method for nanoparticle delivery. This study tests the novel concept that controlling nanoparticle shape by self-assembly can provide passive cell targeting. We tested four different shapes: filomicelles (FMs), bicontinuous nanospheres (BCNs), polymersomes (PSs), and micelles (MCs). These were all self-assembled using fluorescently tagged PEG-*bl*-PPS and tested with the 4T1 tumor model to determine how shape affects uptake by tumor cells and associated immune cells. Nanoparticle uptake was determined by incubating 4T1 cells and healthy splenocytes with nanoparticles for 24 hours in vitro, fluorescently stained for viability and immune cell markers, and analyzed using flow cytometry. To assess immune cell biodistribution *in vivo*, 1 $*10^6$ cells 4T1 cells were injected into the mammary fat pad of 4-9 month old female BALBc mice. Tumor size was measured as cross-sectional area by caliper bidaily. Fluorescently tagged nanoparticles were injected intratumorally when tumors reached 10-15 mm in diameter. Mice were sacrificed 24 hours post intratumoral injection. Tumor, spleen, and inguinal lymph nodes were extracted and dissociated. Immune cells were stained for cell type and viability with fluorescently tagged antibodies and uptake of nanoparticles was determined using flow cytometry. Our data support further investigation of NP shape as a method for passive targeting and drug delivery to tumor and immune cells.

Danielle Cannady
University of Maryland, Baltimore County
Mentor: Dr. Aikaterini Kontrogianni- Konstantopoulos

Impact of the obscurin-PH domain in cell growth, motility and invasion

Obscurins are a family of giant cytoskeletal proteins, which, upon loss lead to cellular transformation and tumor potentiation of breast epithelium via deregulation of various cellular mechanisms. One such mechanism is the interaction between the Pleckstrin homology (PH) domain of obscurins and the p85 regulatory subunit of phosphoinositide-3 kinase (PI3K). Upon loss of obscurins and consequently the loss of their PH domain, the PI3K pathway becomes activated resulting in the upregulation of various downstream proteins that promote motility, growth, and invasion. Specifically, previous studies show that over-activation of PI3K results in conversion of epithelial cells to a more mesenchymal phenotype (epithelial-to-mesenchymal transition), which increases the ability of cells to be motile and invade other tissues. We sought to show that restoration of the obscurin PH-domain in obscurin-knockdown cells results in reversion of these characteristics. To test this hypothesis, we transfected shRNA mediated obscurin knockdown MCF10A cells with an expression vector containing the obscurin PH-domain fused to a myristoylation tag (myr-PH) that ensured its proper targeting to the cell membrane. We confirmed successful expression of myr-PH via Western blot and membrane localization via immunofluorescence. In the future, we will conduct an in vitro scratch assay to examine whether expression of myr-PH suppresses cell motility.

Carina Sclafani
Wheaton College
Mentor: Dr. Alex Drohat

Targeting Apurinic Endonuclease 1 (APE1) Using Fragment Based Drug Design

Apurinic endonuclease 1 (APE1) is a crucial enzyme in the DNA base excision repair (BER) and single strand break repair (SSBR) pathways, that helps to protect against many types of DNA lesions. APE1 has previously been found to be overexpressed in cancer cells, and is thought to hinder treatments that rely on DNA damage to kill cancer cells, such as ionizing radiation (IR) and chemotherapy. As well, it has also been found that inhibition of the APE1 protein in tumor cells increases the cell's sensitivity to such treatments. The amount of APE1 found in tumor cells, then, can be used as an indication to the cell's susceptibility to certain treatments. APE1 has been considered a potential cancer therapy target, and attempts at finding a chemical inhibitor have been made, but there remains no effective inhibitor to date. The aim of this project is to identify potential compounds that inhibit APE1 that could be elaborated to lead compounds, with a long term goal of developing an anticancer drug. The use of fragment based drug design in this project is more beneficial than screening larger compounds, as many smaller compounds may be able to be combined to form new compounds that more tightly bind to the enzyme. Previous screening tests of compounds used were DSF and molecular beacon high-throughput (HT) fluorescent assay to identify potential inhibitors. I was involved in the protein purification process of from recombinant *Escherichia Coli* which was used in molecular beacon assay experiments and other procedures. I performed molecular beacon assays with potential inhibitors, and previously found inhibitors, to find the most promising compounds. As well, I performed assays on gels to confirm findings in my own and other previous beacon assays. Further assays will be performed with successful compounds of varying concentrations to determine the IC50 value of each compound. Future studies should continue searching for potential inhibitors, as well as attempt to locate where the site of the protein compounds bind to using structural biology techniques.

Richa Beher
Univ. of Maryland College Park
Mentor: Dr. Maria Baer

Concurrent Treatment of PARP I Inhibitor and TOP2 Inhibitor Decreases DNA Repair in Acute Myeloid Leukemia Cells with FLT3-ITD

Acute myeloid leukemia (AML), the common form of acute leukemia in adults, is characterized by accumulation of immature white blood cells in the bone marrow. This accumulation prevents normal hematopoiesis. AML cells of 30% of patients have an internal tandem duplication (ITD) of the *fms*-like receptor tyrosine kinase 3 (FLT3). FLT3 plays important roles in hematopoietic stem/progenitor cell survival, proliferation, and differentiation, and ITDs cause its constitutive and aberrant signaling. AML is treated with combination chemotherapy which includes a topoisomerase-2 (TOP2) inhibitor, most commonly daunorubicin (DNR). DNR triggers apoptosis by generating DSBs that harm the integrity of the genome. AML patients with FLT3-ITD relapse after a short disease-free survival following chemotherapy and transplantation. Previous studies have shown increased DNA damage resulting in double-strand breaks (DSBs), impaired DNA repair by homologous recombination (HR) and non-homologous end joining (NHEJ), and upregulated error-prone alternative (Alt-)NHEJ repair in cells with FLT3-ITD. Therefore, inhibition of poly(ADP-ribose) polymerase (PARP), an enzyme involved in Alt-NHEJ, may decrease DNA repair in cells with FLT3-ITD. In this study, we demonstrate that the PARP inhibitor talazoparib sensitizes cells with FLT3-ITD to the effects of the TOP2 inhibitor DNR through increased DNA damage. The Ba/F3-ITD and MOLM-14 cell lines, with FLT3-ITD, were treated with DNR or talazoparib alone or in combination. Apoptosis was measured by Annexin V staining, detected by flow cytometry, and by PARP cleavage, detected by immunoblotting. DNA damage was measured by γH2AX expression, detected by immunoblotting. Concurrent treatment caused increased γH2AX expression and Annexin V staining and PARP cleavage, compared to single drug treatments. Increasing the efficacy of chemotherapy while decreasing toxicity by targeting altered signaling pathways associated with genetic abnormalities in AML blasts appears to be a promising approach to improving patient outcomes.

Ryan O'Donnell
Loyola University Maryland
Mentor: Dr. Xuefang Cao

CD27 mediated regulation of allogeneic T cell response

Allogeneic hematopoietic cell transplantation is a potentially curative therapy for hematologic malignancies, including leukemia and lymphoma. Approximately 20% of patient deaths following allogeneic hematopoietic cell transplant are due to Graft Versus Host Disease (GVDH). GVHD is caused by the immune activation of donor cells recognizing the recipient cells as foreign. T cells chiefly function in cell-mediated immunity and are primarily responsible for the beneficial graft-versus-tumor (GVT) effect, however they also cause the adverse GVHD effect. Recent studies have shown that CD27, a T cell surface protein in the tumor necrosis factor (TNF) superfamily, plays a crucial role in cellular immune response through CD27-CD70 co-stimulation. This is because CD27 is largely regulated by CD70 expression, a cell surface protein on antigen presenting cells (APC) as well as activated T cells. We hypothesize that CD27 activation skews bone marrow cell development and suppresses allogeneic T cell response. We developed an in vitro model using a bone marrow dendritic cell (BMDC) culture and an agonistic CD27 antibody to stimulate CD27 activation. The BMDCs were then used to model allogeneic T cell response in a mixed lymphocyte reaction (MLR). The function of the MLR-activated T cells was assessed by their tumor killing activity against leukemia cells as a readout of GVT effect. Our data supports CD27-CD70 co-stimulation as a pivotal regulator of allogeneic T cell response. Further study is needed to assess how this pathway impacts GVHD and GVT effect in vivo.

Olivia Konen
Towson University
Mentor: Dr. A-Lien Lu-Chang

Determination of the Consequences of Disrupting Interaction Between MYH Repair Enzyme and the Checkpoint Clamp

Exposure to reactive oxygen species and radiation leads to DNA damage that can compromise genomic integrity. 8-oxo-guanine (GO) is one of the most frequent and highly mutagenic oxidative lesions because it mispairs with adenine during DNA replication. The MYH glycosylase increases replication fidelity by removing adenines misincorporated opposite GO. Individuals with a mutation in the *hMYH* gene are at an increased risk of developing colorectal cancer. MYH-directed base excision repair (BER) is tightly coordinated with the DNA damage response (DDR) in order to maintain genomic stability and cell survival. The 9-1-1 complex (Rad9, Rad1, and Hus1 heterotrimer complex), a DDR sensor, is essential for cell viability and development and is proposed to provide a platform to coordinate BER processes to avoid the accumulation of toxic intermediates. The major Hus1-binding site is localized to residues 295–350 (interdomain connector, IDC) of hMYH. The goal of this project is to interrupt MYH-Hus1 interaction by overproduction of IDC peptides. We propose that the overproduced IDC peptide will inhibit Hus1 binding to hMYH, increasing cell sensitivity to oxidative stress and apoptosis. We amplified the DNA fragments encoding IDC peptides (residues 295-350) of hMYH using plasmids containing wild-type (WT, V^{315}/Q^{324}) and mutant (V315A and Q324H). After digestion with XhoI and EcoRI, the PCR products were ligated into cleaved pEGFP-N1. After verification by restriction digestion and DNA sequencing, the clones were transfected into HeLa cells to express the GFP-fusion proteins. We observed that transfected cells contained green fluorescence. We will perform experiments to show physical interactions between GFP-IDC (WT, V315A, and Q324H) with Hus1 and to demonstrate the biological consequences of expressing IDC in HeLa cells. Because expression of SpIDC renders fission yeast *S. pombe* cells more sensitive to H_2O_2, we expect that expressing GFP-IDC (WT), but not mutant IDCs, will render HeLa cells more sensitive to H_2O_2 than control cells. Our results suggest that disrupting the links between BER and DDR proteins may provide a new strategy for cancer therapy.

Lyra Morina
University of Maryland College Park
Mentor: Dr. William Jackson

Contribution of Starvation Factors to Autophagic Signaling and Degradation

Autophagy is a key mechanism for the breakdown and recycling of cellular materials. In times of stress, cells can show increased levels of autophagy. Although positive for normal human cells in moderation, autophagy can be “hijacked” by cancerous cells to promote over-proliferation in nutritionally deficient tumor microenvironments. The aim of this project is to define the contribution of amino acid or carbon source starvation to autophagic induction and degradation. Through immunoblot analysis of autophagy markers LC3 and SQSTM1, the effect on autophagy of varying glucose or amino acid concentrations for five hours was determined. As expected, decreasing the level of amino acids resulted in a dose-dependent increase in autophagy. However, decreasing glucose levels did not show a significant difference in autophagy. Overall, this shows signaling the induction of autophagy is more sensitive to amino acid starvation than glucose starvation. One possibility is that glucose starvation induces autophagy at a slower rate and requires a longer incubation time. Determining the specific concentration of amino acids and glucose for optimal starvation and induction of autophagy is also important as it will allow for standardization of starvation in future autophagy studies. Additionally, it would be beneficial to determine which specific amino acids play a greater role in the induction of autophagy. By characterizing the fundamental, cellular, autophagic response to metabolic stressors, we are one step closer to understanding the operations of cells in tumor microenvironments.