Spring 2023
Developmental Awards

Tier 1 – Basic Science

PI: Sean Clark-Garvey, MD, MPH, Medical Oncology Fellow, Lineberger Comprehensive Cancer Center

Project Title: Exploring FGFR3 inhibition as a sensitizing agent to Nectin-4 ADC therapy in urothelial carcinoma and investigation of associated changes to the tumor microenvironment.

Abstract
In 2022, there will be an estimated 81,180 new cases of bladder cancer in the United States, which will result in an estimated 17,100 deaths. There have recently been important advances in the treatment of bladder cancer, but the prognosis remains particularly poor for those with advanced stages of the disease. Our lab has been trying to understand why some patients don't respond to certain treatments and trying to determine if there are ways to improve responses to therapy. We have now found that in a specific subtype of bladder cancer there are higher levels of two proteins, FGFR3 and Nectin-4, both of which can be targeted by some of the newly approved drugs for bladder cancer. Additionally, we found that by using a drug called erdafitinib (which halts the activity of FGFR3) we see an increase in the other protein, Nectin-4. This is an indication that the two drugs may work well in combination. In our proposed research we first plan to understand more about why levels of Nectin-4 go up when we halt FGFR3 activity. We then plan to study the combination of erdafitinib with a drug that targets Nectin-4 to see if this is an effective combination. We will also evaluate this combination with immunotherapy, which is another effective treatment for bladder cancer. This portion of the study will be carried out in mice. We will also monitor for safety do some additional studies to help understand how the different combinations of drugs work. If successful, this work may have implications for further investigation and treatment in patients.

Tier 1 – Basic Science

PI: Lee Graves, PhD, Professor, Pharmacology; Justin Milner, PhD, Assistant Professor, Microbiology and Immunology; and Aadra Bhatt, PhD, Assistant Professor, Center for Gastrointestinal Biology and Disease

Project Title: Small Molecule ClpP Activators to Improve Immune Therapies for TNBC

Abstract
We are studying a novel class of anti-tumor agents, based on the chemical structure of ONC201 and the more recently developed TR compounds (Madera Therapeutics, Cary NC), for their effects on triple-negative breast cancer (TNBC). Through UCRF funding, we were able to demonstrate that the molecular target for these small molecules was an unexpected mitochondrial protease ClpP. This research changed the direction of the field and provided the first insight into how target modulation (ClpP activation) could impact cancer growth. The focus of this proposal is to now study the specific effects of pre-clinical lead compounds TR57/TR107, on mechanisms of immune modulation. We have preliminary evidence that ClpP activation, through effects on mitochondrial stress, alerts the immune system through changes in a tumor cell surface protein (calreticulin, CALR). Moreover, we show that TR compound-treated cancer cells become "sensitized" to killing by natural killer (NK) cells. To investigate this, we have established a collaboration with Drs. Milner and Bhatt to examine the tumor/immune component response to small molecule ClpP activators. We will pursue the unique possibility that tumor cells can be "primed" for killing by NK cells and will determine if this is contributing to tumor reduction in mice. Thus, we are poised to elucidate fundamentally essential insights into the anti-tumor mechanisms of these compounds and to develop innovative new strategies for the treatment of TNBC.

Tier 1 – Basic Science

PI: Brian Strahl, PhD, Professor, Biochemistry and Biophysics; Abid Khan, PhD, Research Associate; Nate Hathaway, PhD, Associate Professor and Facility Director, UNC CRISPR Screening Facility and Eshelman School of Pharmacy; and Brian T. Golitz, Manager – CRISPR Screening Facility – Automation & Robotics, Eshelman Institute for Innovation

Project Title: Identification of chromatin modifiers that regulate nuclear morphology and genome stability

Abstract
One of the defining features of a cancer cell is a grossly misshaped nucleus, the region where our genetic material is stored inside the cell. Proteins called "epigenetic modifiers" regulate how the information stored in our DNA is accessed and interpreted. The genes that encode epigenetic modifiers are often mutated in cancer, leading to changes in nuclear morphology and genetic instability—a hallmark of all cancer cells. Yet, how mutations in these epigenetic modifiers causes misshaped nuclei and genetic instability is not well understood. Our recent study on one such epigenetic modifier, called SETD2, has led us to reimagine how the epigenetic modifiers may be functioning to maintain nuclear morphology. We found, an unexpected role for SETD2 during cell division to control the morphology and stability of the nucleus. Perturbing SETD2 leads to grossly misshaped nuclei and genetic instability that we believe is how it contributes to cancer progression. However, we have evidence that SETD2 does not do this alone. Rather, it may be that a number of other epigenetic modifiers, similar to SETD2, also have this role – a result that if true, would shed new light on how epigenetic regulators contribute to cancer. Thus, we seek Tier 1 funding to ask the question: how many other chromatin regulators contribute to the prevention of nuclear abnormalities and genome instability? To answer this question, we will be using cutting-edge CRISPR technology to perform a gene deletion screen of 311 epigenetic modifiers, where we can delete individual epigenetic modifier genes and assess the impact on nuclear morphology and genetic abnormalities through microscopy. This focused study stands to uncover an unappreciated mechanism by which epigenetic modifiers help maintain genetic integrity that would lead to new avenues of research that one day could provide new therapeutic approaches to treating cancer.

Tier 1 – Clinical/Translational

PI: Theodore Yanagihara, MD, PhD, Assistant Professor, Radiation Oncology

Project Title: Development of a circulating tumor DNA fragmentomics assay for monitoring treatment response in patients with hepatocellular carcinoma

Abstract
Hepatocellular carcinoma is the most common type of cancer that originates in the liver. There are many treatments available for hepatocellular carcinoma, but unfortunately, new cancers will often grow in areas of the liver that were not treated. Keeping track of cancers in the liver requires frequent blood tests and scans, such as MRI scans, which can be costly and difficult for many patients to undergo. In some cases, patients are not able to have all these tests, or they have to travel to a specialized center to have them performed. We are interested in developing new ways of detecting liver cancers that can 1) find cancers earlier and 2) reduce the testing burden on patients. A new technology has been developed that allows scientists to measure cancer DNA in a patient's blood and doctors are trying to learn if this could be used to help patients with a variety of cancer types. In this study, we will test this new technology to see if it is successful at detecting cancer DNA in patients with hepatocellular carcinoma. In addition, we would like to see if the cancer DNA disappears from the blood after a patient is treated with targeted radiation for their liver cancer. If this study is successful, it is possible that this technology could be used to help future patients and we can test it in a larger study to determine the best way to use it. By performing this series of careful studies, patients and their doctors could be able to look for new liver cancers and check the response to treated cancers, all with a blood test.

Tier 1 – Clinical/Translational

PI: Jessica Benjamin-Eze, DO, Clinical Fellow, Hematology/Oncology

Project Title: Pilot Study: Measuring changes in body composition and physical function in patients with childhood cancers

Abstract
Children with cancer frequently experience muscle loss, weakness, and impaired physical function due to cancer and its treatments. Parents worry if their child will regain lost muscle and strength after finishing cancer treatment. Additionally, these negative physical changes may become longstanding and contribute to the earlier development of aging-related medical problems (diabetes, high blood pressure, high cholesterol) and risk of earlier death. Currently, there is limited data in the childhood cancer population about when these unwanted changes happen, how long these changes last, and how to prevent these changes. The main goal of our study is to determine what measures of muscle mass and physical function (strength, mobility, endurance) are feasible for pediatric patients during active cancer treatment. Our second goal is to gain an initial understanding of how muscle mass, strength, mobility, and endurance change during cancer treatment. Identifying loss of muscle mass or physical function during treatment will allow for earlier intervention to prevent these changes and their associated harmful outcomes. As an innovative approach, we are testing a newer, non-invasive way to measure muscle mass (D3-Creatine dilution). This method can be completed in many settings (clinic, home, hospital) and may allow for more consistent and accurate tracking of muscle loss experienced by children during their cancer treatment. The initial data generated from this project will lay the foundation for larger studies with the long-term goal of testing exercise and nutrition interventions to prevent these problematic changes. Appropriate interventions will improve quality of life during cancer treatment and survivor health.

Tier 1 – Clinical/Translational

PI: Jill Dowen, PhD, Associate Professor, Biochemistry and Biophysics

Project Title: Precision Detection of Structural Variants In AML

Abstract
Leukemia is a cancer of the bone marrow and blood that results in uncontrolled proliferation of progenitor cells. Sequencing technologies allow for precise detection of mutations and chromosomal rearrangements in cancer cell genomes. Surprisingly, these sequencing approaches have revealed that children with leukemia acquire a very different set of mutations than adults who develop leukemia. The molecular signatures of childhood and adult leukemias indicate a completely distinct molecular origin of disease in patients of different ages.

Clinical tests for detection of cancer mutations (including base pair changes, deletions, duplications, inversions, and translocations) usually include 1) DNA FISH (fluorescent in situ hybridization) to detect known sequence changes, 2) karyotyping, which can only detect very large chromosomal changes, or 3) exome sequencing of a limited panel of genes implicated in disease. All of these diagnostic methods have limitations and may not detect every genetic lesion possible in a patient. We propose to use Hi-C/Micro-C to detect all cancer mutations (including structural variants) in leukemia patient samples to 1) demonstrate the utility of this technique as a diagnostic approach, 2) identify any and all mutations present in a patient sample (rather than the candidate mutations being assayed in current tests) to understand the complete mutation burden, 3) determine whether mutations are found in healthy individuals to explore their association with disease state and natural aging, and 4) understand how mutations effect chromosome structure.

Our recent work has shown that cancer mutations in the cohesin complex re-wire the spatial folding pattern of DNA in the nucleus and cause gene expression changes in cells. We propose to apply our approach to investigate the origins of pediatric AML. Our innovative ideas and methods will improve diagnosis of disease

and elucidate the molecular and cellular consequences of common sequence variation in cancer. The knowledge generated from the proposed project could lead to improved diagnosis and therapeutic approaches based on a patient's clinical genomics signature.

Tier 1 – Population Science

PI: Di Wu, PhD, Associate Professor, Adam School of Dentistry and Assistant Professor, Biostatistics

Project Title: Drug Repurposing for Head and Neck Cancer using Genomics Data Integration and Electronic Health Records

Abstract
Head and neck cancer(s) (HNC) are deadly cancers for which there are limited effective treatments. Current treatments involve surgeries to excise tumors, radiation, and therapies using medication like chemotherapy. The current process for discovering new drugs and medications for HNC treatment is costly and time consuming. Drug repurposing is to ask whether existing drugs that were indicated for other diseases can also be used for another specific type of diseases. It is safer and more cost-effective. for drug repurposing of HNCs, we will use two most efficient computational methods either based on increasing knowledge of the disease mechanism, such as genomic data, or using evidence-based strategy by utilizing the electronic health records (EHR). In genomic-based method, we will integrate multi-omics data from large cohorts to find the repurposed drug candidate from FDA approved drugs. In EHR-based method, we will use both tumor recurrence and recurrence-free survival as evaluation of drug effects in EHR. Regarding HNC may include multiple subtypes due to multiple different sites in head and heck and different tumor driver factors (such as HPV+/-), we will carefully define the predicted tumor recurrence by applying the best available machine learning tools combined with expertise inputs. Our previously developed EHR-based drug repurposing prediction model will ensure drug candidates using the EHR of large HNC cohort of ~7,000 patients at NC we recently extracted. We expect wet lab validation of drugs in cell lines and external computational validation at other health system after the completion of the proposed work.

Tier 2 – Basic Science

PI: Yevgeny Brudno, PhD, Assistant Professor, Biomedical Engineering

Project Title: Same-Day CAR T Cell Therapy for Dogs

Abstract
Chimeric Antigen Receptor T (CAR T) cells has revolutionized the treatment of cancer, particularly leukemia and lymphoma. Despite the excitement surrounding this remarkable innovation, there are two vexing problems with CAR-T therapy: cost and toxicity. Bringing this therapy to resource-poor settings, such as the developing world, and to veterinary oncology, is currently impossible due to extremely high costs ($500,000) and time (4-8 weeks) needed for manufacturing CAR T cells. Additionally, improvements in CAR T cells are hampered by absence of animal models that realistically mimic human disease. For example, mouse tumor models are often done in otherwise healthy animals and in animals that completely lack immune systems. Mice also do not show the side effects of CAR T cell therapy that are so common in humans. There is growing recognition that spontaneous canine cancers represent an important, untapped means to test immunotherapy advances. Our labs propose to combine our expertise in dog immunotherapy (Hess and Mariani), biomaterial science (Brudno), and CAR T cell therapy (Dotti) to develop biomaterial scaffolds that will reduce CAR T cell manufacturing times from 14-30 days to a single day, and to apply this breakthrough technology to dog cancers. This collaborative endeavor, which combines our multiple fruitful, ongoing research programs into a single whole, will ultimately lead to cost-effective, low-toxicity CAR-T cell therapy. Together, we hope to create an affordable and potent therapy for dog owners as well as develop the next generation of therapeutics for human treatment.

Tier 2 – Basic Science

PI: Owen Fenton, PhD, Assistant Professor, Eshelman School of Pharmacy, and Paul Armistead, MD, PhD, Associate Professor, Medicine

Project Title: Novel Tumor Antigen-Specific RNA Nanoparticle Vaccines In Reconstituted Immune Systems

Abstract
The most successful COVID vaccines use a new a technology called, mRNA lipid nanoparticle vaccines. These vaccines were found to be very safe and effective in preventing COVID infection, and they are very easy to modify for other purposes. In this research proposal, we will use the basic components that are used to produce mRNA lipid nanoparticle vaccines and modify them so that they can be used to treat acute myeloid leukemia (AML) and melanoma.

Tier 2 – Basic Science

PI: Ronit Freeman, PhD, Associate Professor, Applied Physical Sciences; and Shawn Hingtgen, PhD, Associate Professor, Pharmacoengineering and Molecular Pharmaceutics and Associate Professor, Neurosurgery

Project Title: UberCell: Programmable peptide-cell delivery drives cancer therapy into high gear

Abstract
UberCell drives cancer therapy in a new direction. Just like the ride sharing program, UberCell gets the "passenger" therapeutics riding on a "driver" tumor homing cell, to the right target, at the right time. Having engineered, characterized, and demonstrated efficacy in vivo with our first UberCell therapy, NSC-TRAIL, we are now seeking to expand the platform as well as develop our lead asset. First, we will leverage our panel of cell surface tethers to optimize key factors including tether length and geometry, surface accessibility, conjugation kinetics, loading doses, responsiveness, and ability of UberCell to couple and decouple NSC-payload fusions in presence of tumors. Assembly, activity, and efficacy of this new therapeutic approach will be tested in vitro as well as in vivo using kinetic imaging and molecular assays. Next, we will test combinations of NSC modified with strategic payloads (TRAIL; Chemo; Cytokines) to steer cell therapies and tumor kill towards the most efficacious therapeutic regimens. Lastly, we will advance the most efficacious uber-payload combination and test its ability to improve the tumor response. In this iterative process, we will create an optimized strategy that can achieve potent and lasting tumor kill and is positioned for translation into human patients.

Tier 2 – Basic Science

PI: Sam Lai, PhD, Professor, Eshelman School of Pharmacy, Division of Molecular Pharmaceutics; Pietro Dotti, MD, Professor, Immunology; and Gaorav Gupta, MD, PhD, Assistant Professor, Radiation Oncology

Project Title: Engineering bispecific binders against Siglec-15 and TGF-Beta for enhanced therapy and prevention of metastasis in triple negative breast cancers

Abstract
Triple-negative breast cancer (TNBC) is the most aggressive form of breast cancer that frequently culminates in deaths from metastasis, with more than 40,000 deaths each year. A hallmark of TNBC is its local environment, termed tumor microenvironment (TME), that strongly suppresses the ability of the immune system in killing cancer cells. This makes overcoming the immunosuppressive TME essential for effective immunotherapy. Clinical benefit to date with molecules that target any single immune suppressive pathway has been limited, likely because TNBC has evolved diverse mechanisms to evade immune cell killing. This suggests that effective management of TNBC must involve a multifaceted approach to manage and overcome the immunosuppressive TME. Towards that end, we have identified Siglec-15 (S15) as a novel target for management of TNBC. We designed a bispecific molecule that bind both S15 and TGF-β, another protumor molecule secreted by TNBC cells to suppress immune response, and found it highly effective at limiting tumor growth, reducing immune suppression within the tumor, and eliminate metastasis. Here, we seek to optimize our engineering of proteins

and antibodies to better bind S15 and TGF-β, and evaluate their potencies in vivo. By improving the binding affinity or reducing the size of our molecules, we may be able to achieve even more uniform distribution within the tumor, and/or more effectively inhibit immune suppression thus resulting in more effective therapies. If successful, results from these studies will strongly support further development for a novel therapeutic target for TNBC and other cancers that overexpresses S15.

Tier 2 – Basic Science

PI: John P. Morris IV, PhD, Assistant Professor, Pharmacology; and Kirsten L. Bryant, PhD, Assistant Professor, Pharmacology

Project Title: Identifying determinants of PDAC heterogeneity that predict response to dual ERK MAPK and autophagy inhibition

Abstract
Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer deaths in the US, with a dismal 12%, 5-year rate of survival. Over 90% of PDAC patients have a mutation in the gene KRAS. Oncogenic KRAS signaling leads to overactivation of a series of proteins that result in uncontrolled growth and proliferation. Because so many patients harbor overactivation of this signaling pathway, developing therapeutics that inhibit this pathway has been a focus of cancer researchers for decades. While current inhibitors of this pathway do slow the growth of cancer cells, resistance often develops. One method of resistance that PDAC cells employ is to upregulate metabolic pathways to increase growth. We found that when treated with inhibitors of the RAS pathway, PDAC cells upregulate a metabolic process called autophagy, which literally means cellular self-eating. If we inhibit the autophagy pathway and the RAS pathway simultaneously, we can further inhibit PDAC grow. This strategy was discovered by our group as well as two others in 2019. Today there are multiple clinical trials open in the United States and Europe testing the combination of different inhibitors of the RAS pathway with inhibitors of the autophagy pathway. However, early preclinical reports show that while patients do initially respond to this therapy, resistance does arise over time. Therefore, we want to better understand how PDAC evolves to be resistant to this therapy. We have developed models that represent different stages of PDAC evolution. When we tested our inhibitor combination in these models, we identified some that were responsive and some that were not. This proposal is focused on better understanding the non-responsive models to develop ways to shift them back into the responsive state. The ultimate goal of this study is to better understand how resistance to therapies evolve and ultimately generate improved therapies for PDAC patients.

Tier 2 – Basic Science

PI: Otto Zhou, PhD, Professor, Physics and Astronomy

Project Title: Improving imaging guidance in radia0on therapy for be6er cure of cancer

Abstract
Radiation therapy (RT) is one of the most effective treatment modalities for cancer treatment. Image-guided radiation therapy (IGRT) uses high resolution “real time” imaging technologies to visualize the tumor inside the patient at the time of treatment to enable precise delivery of radiation dose to tumors while minimizing damage to the normal tissues. Cone beam CT (CBCT) is the most commonly used imaging method for IGRT today. The current CBCT however has several intrinsic limitations, including: 1) not able to “see” the tumor surrounded by tissues of similar density; and 2) not able to accurately measure/calculate the radiation dose delivered to the patient. We recently demonstrated a new type of CBCT that can potentially overcome these limitations. In dental and head imaging studies, we have demonstrated that the new technology significantly improves the performance of the CBCT. The purpose of this project is to evaluate whether the drastically improved performance observed can be achieved under the conditions and environment required for IGRT. If successful, the new technology can lead to significantly improved accuracy and speed for tumor imaging and improve the accuracy of dose delivery.

Tier 2 – Clinical/Translational

PI: Kathleen Conway Dorsey, PhD, Associate Professor, Epidemiology, and Nancy Thomas, MD, PhD, Distinguished Professor, Dermatology

Project Title: Visium spatial transcriptional characterization of melanoma cell states and intratumor heterogeneity in the poor-prognostic primary melanoma subtype exhibiting the CpG island methylator phenotype (CIMP)

Abstract
Melanoma of the skin is a highly aggressive cancer with a tendency to metastasize early. A major challenge in treating melanoma lies in its high cellular 'plasticity' (ability to change characteristics) driven primarily by the process of 'phenotype switching' in which melanoma tumor cells reversibly switch back and forth between a proliferative/differentiated (more normal-like) melanocytic cell state or an invasive/dedifferentiated mesenchymal state. Intermediate cell states may also exist along with rare dedifferentiated, therapy-resistant tumor subpopulations with unique stem-like properties. Such plasticity produces mixtures of different cell states within each tumor (intratumor heterogeneity) that is thought to underlie the aggressive nature of melanoma characterized by immune exclusion, therapeutic resistance, metastatic spread and poor outcomes. We recently identified a group of primary melanomas exhibiting a 'CpG island hypermethylated phenotype (CIMP)' with poor survival compared with melanomas exhibiting low methylation/LM. Bulk tumor mRNA expression analyses yielded conflicting data indicating CIMP tumors are proliferative/differentiated but also express the SOX2 stem cell dedifferentiation marker. In this study, we will undertake the new Visium transcriptional profiling to spatially map >18K gene expression and coexpression patterns at near-single cell resolution across early primary invasive melanoma tissues with CIMP (n=20) or LM (n=20) methylation subtypes. Our goals are to deconstruct the complex mixtures of cell types and cell states, including from rare stem-like cells, residing within CIMP and LM melanomas, assess pathways underpinning these cell states, quantify intratumoral heterogeneity, and analyze spatial relationships among melanoma, immune and stromal cell types/states that may contribute to patient survival differences.

Tier 2 – Clinical/Translational

PI: Andrew Satterlee, PhD, Research Assistant Professor and Associate Director of the Brain Slice Technology Program, Eshelman Institute for Innovation, and David Kram, MD, Associate Professor, Pediatrics

Project Title: A feasibility study of a novel patient-derived explant platform for precision oncology in children with CNS tumors

Abstract
Our team is developing an innovative platform where we test the patient's actual tumor tissue after surgical removal. We can treat each patient's removed tumor with a panel of drugs and get functional tumor-killing readouts that suggest drugs which may be effective at killing leftover tumor in the patient. All fast enough to guide care. Many current methods struggle to test living brain tumor tissue outside the body, but our innovative approach builds on 20 years of data development, and begins by rapidly slicing fresh brain tissue from rats like a loaf of bread. The key is that the living brain slice captures the tumor just like it was in the patient. Using over two-dozen tumor tissues collected straight from the operating room and transferred to our research lab, we've shown the slices allow engraftment of tumors rapidly and efficiently. Critically, we also showed the tumor on the slices looks very close to the way it does in the brain of the patient, down to even the genomic level. We have tested a large number of different drugs, from those already used in human patient treatment to novel next-generation compounds. We've also created a color-coded scoring system that's easy for clinicians to read which drugs worked and which drugs didn't fare so well. We now propose to utilize this Development Award to support a critical next step: First-in-human clinical trial. With a focus on children with brain cancer and using our protocol that is nearly approved at UNC, we will first test our ability to take a tumor from the operating room where it was freshly removed from a child's brain, grow the tumor on living brain slices, test a panel of drugs chosen by the treating oncologist, and provide a drug score for each agent generated from watching the tumor live or die on brain slices. All within the clinically useful timeframe

of 28 days. Using this tissue and information collected from the patient's medical records, we will also begin to define the predictive power of the brain slice platform by correlating the response of tumors on brain slices with (1) genetic markers that suggest if the patient's tumor should respond to treatment, and (2) the response of the child to the same drug during their clinical care. Lastly, we will investigate a new multi-round testing approach where the results from the previous round of testing are used to rule different drugs in or out for the next round of testing. Once complete, our outstanding team of researchers and clinicians will have generated results that are essential to advancing this important platform into human patients, where it can help match each child to the treatment that is most effective against their own tumor, and start to truly improve the outcomes for children suffering from brain cancer.