Fall 2022
Developmental Research Awards

Tier 1— Basic Science

PI: Jose Martinez, MD, PhD, Hematology Fellow

Project Title: Defining the U2AF1-driven translatome to understand therapy resistance and identify therapeutic targets in AML

Abstract
Acute myeloid leukemia (AML) remains a deadly cancer with a 5-year overall survival of 30.5%. Developing therapies targeting specific AML mutations may improve patient survival. Over the past decade, several mutations in RNA-associated pathways have been linked to hematologic cancers. However, unlike well-studied mutations in tumor suppressors (TP53) and oncogenes (KRAS, NRAS), very little is known about how mutations in RNA regulatory factors contribute to leukemia development and therapy resistance. Mutation of the spliceosome factor U2AF1 carries a very poor prognosis due to a high association with chemotherapy resistance. Recent studies suggest that mutation of U2AF1 disrupts RNA processing pathways including mRNA translation, and non-sense mediated mRNA decay, which are downstream of its known function as an RNA splicing regulator. However, previous research has only focused on characterizing the changes in mRNA composition, or the transcriptome, associated with U2AF1 mutation in AML. Evasion of NMD, tumor-specific RNA splicing, and translation of novel open reading frames, are shown to produce tumor specific antigens in a variety of cancers but remain unexplored in AML. We hypothesize that mutation of U2AF1 in AML induces tumor specific translation of aberrantly spliced transcripts which can be targeted for tumor-specific antigen discovery. The objectives of this proposal are: 1) to characterize the U2AF1-mutant associated leukemia translatome by applying ribosome profiling to leukemia derived cell lines and human samples, and 2) to discover and validate U2AF1-mutant associated TSAs by carrying immunopeptidomics in leukemia cell lines.

Tier 1— Basic Science

PI: Justin Milner, PhD, Assistant Professor, Microbiology & Immunology

Project Title: Epigenetic regulation of the tumor immune microenvironment

Abstract
Despite the curative potential of cancer immunotherapies, most patients do not respond to current modalities. The composition of immune cell types within the tumor microenvironment critically influences immunotherapy success and overall tumor progression. However, therapies designed to deliberately and comprehensively manipulate the overall composition of the “tumor immune microenvironment” (TIME) remain limited in number and efficacy, in part due to the extensive diversity of immune cell (sub)types in the TIME and the myriad of complex signals controlling their localization and activity. This gap in knowledge and treatment options presents an exciting opportunity to improve the immunotherapy landscape. We propose to address this gap by leveraging recent technical advances to create a high-throughput genetic screening pipeline that will allow us to perturb expression of many genes in parallel in all cells of the TIME in vivo and dissect the resulting effects through an integrative single-cell genomics approach. This transdisciplinary immunotherapy discovery platform, TIME-Map,

builds on our expertise in genetic screens and single-cell omics, but would massively accelerate progress relative to current approaches that explore the TIME gene-by-gene or cell-by-cell. This LCCC Developmental Award application seeks to establish key tools and evidence of feasibility for TIME-Map through performing a small-scale, multi-omic single-cell genetic screen to identify potential therapeutic targets controlling the TIME in a preclinical model of triple negative breast cancer. In summary, we aim to develop a new immunotherapy pipeline that will reveal fundamental tumor immunology and facilitate design of new therapeutic strategies for effective and deliberate modulation of the TIME.

Tier 1— Basic Science

PI: Denis Okumu, Postdoctoral Research Associate, Pharmacology

Project Title: Development of a diagnostic assay to predict trastuzumab deruxtecan efficacy using targeted proteomics of ERBB receptor tyrosine kinases [ERBB1 (EGFR), ERBB2 (HER2), ERBB3 (HER3) ERBB4 (HER4)]

Abstract
Triple-negative breast cancers (TNBCs) are a heterogenous tumor group that vary in prognosis and sensitivity to chemotherapy. Targeted kinase therapies (MEKi) are not durable and relapse often occurs due to adaptive kinome remodeling characterized by upregulation of receptor tyrosine kinases. Anti-PD-1 in combination with chemotherapy is FDA-approved, but many patients respond poorly just as with chemotherapy alone. CAR-T cells targeting TNBC-specific antigens are in development. Thus, a critical unmet medical need exists for effective treatment of TNBCs. Recent evidence with the Destiny-Breast04 Trial showed targeting HER2-low expression in HER2-low metastatic breast cancer patients using trastuzumab deruxtecan (T-DXd) is clinically superior to chemotherapy. Patients within this cohort responded to T-DXd with nearly 3-fold longer PFS (8.5versus 2.9 months) and > 2-fold longer OS (18.2 versus 8.3 months) compared to chemotherapy alone. c\Current methods of measuring HER2 abundance in clinical samples are insufficient to predict response to trastuzumab deruxtecan in HER2-low cancers. We have developed a targeted proteomics-based approach, SureQuant/PRM, for absolute quantitation of HER2 attomole protein abundance requiring only ~10ug of crude protein lysate input, suitable for use with patient derived organoids and limiting clinical samples including core needle biopsies and fine need aspirants. We will quantify HER2 expression levels in a panel of TNBC cell lines and patient derived organoids and determine the threshold for HER2 expression defining sensitivity to trastuzumab deruxtecan in models of TNBC. These studies will provide the foundation for development of a diagnostic CLIA assay to guide the clinical use of trastuzumab deruxtecan.

Tier 1— Basic Science

PI: Hong Yuan, PhD, Professor, Radiology

Project Title: Developing FAP targeted theranostic agents for brain metastases

Abstract
Fibroblast-activation protein (FAP), a transmembrane serine protease, reportedly has high expression in over 90% epithelial tumor, including lung cancer, breast cancer, and many other aggressive tumor types. It is rarely expressed in healthy normal tissue, making it a promising target candidate for cancer diagnosis and treatment. Our team has recently constructed a new Cu-64 labeled FAP inhibitor, 64Cu-Sar-FAPi, as imaging ligand for FAP imaging. Preliminary study of PET imaging with 64Cu-Sar-FAPi in an orthotopic brain tumor mouse model showed high tumor-to-brain ratio, which remained high even at 24 h post injection. There was little uptake in normal brain and very low in liver and kidney parenchyma.

The preliminary data demonstrated that this new FAPi compound could be an excellent treatment vehicle once it is labeled with therapeutical radionuclides, such as Cu-67 (beta emitter, 62 h half-life), as a targeted treatment option for brain tumor. With its low normal brain uptake and wide expression in lung and breast cancer (the top two cancer metastasized to brain), we would like to prioritize the FAPi-based theranostic development for brain metastasis. Hence, this proposal is aimed to develop FAP targeted theranostic agents, in particular, using the 64Cu/67Cu pair, for managing brain metastases. 64Cu and 67Cu are chemically identical, thus the information on radiolabeling and biodistribution are interchangeable between the two agents. Cu-67 has the preferred radioactive decay half-life (2.6 days) matching with the FAPi biological retention scheme, and easier for patient management. Cu-67 also has slightly higher beta energy level compared to the commonly used beta emitter Lutetium-177, thus potentially offers better treatment outcome. In addition, the sarcophagine cage chelator provides better labeling stability for copper. The proposed project will optimize the radiolabeling procedure and evaluate the stability, specificity, and biodistribution of the 64/67Cu-Sar-FAPi in an animal model of brain metastasis. We will then assess the therapeutical efficacy from 67Cu-Sar-FAPi in vivo in the brain metastasis model. If the results are promising, the pilot study will open a new direction of developing 64Cu/67Cu radionuclide-based imaging and treatment approach for brain metastasis and many other cancer types.

Tier 1— Basic Science

PI: David Zaharoff, PhD, Associate Professor, Biomedical Engineering

Project Title: Reversing tumor-infiltrating T cell exhaustion with localized IL-17

Abstract
Interleukin-10 (IL-10) is a 18kDa alpha helical cytokine with seemingly paradoxical biological activities. On the one hand, IL-10 exhibits anti-inflammatory properties via inhibition of antigen presenting cells, T cell activation and inflammatory cytokine production. On the other hand, IL-10 enhances interferon-gamma production by activated CD8+ T cells and increases the proliferation and activation of exhausted tumor infiltrating lymphocytes. These effects have led to significant antitumor responses in preclinical models. Unfortunately, these antitumor responses were not duplicated in recent clinical studies employing daily systemic injections of pegylated IL-10 versus advanced lung cancer. Given the short half-live of IL-10 and the poor uptake of systemically administered cytokines in tumors, the aforementioned clinical failure was likely due to poor delivery of IL-10 to the tumor microenvironment. This pilot project will test the ability of a novel biopolymer-based hydrogel to deliver and sustain high concentrations of IL-10 in the tumor microenvironment following intratumoral injection. This localized IL-10 approach is expected to increase the activity of exhausted CD8+ T cells within injected tumors while reducing the accumulation of immunosuppressive regulatory T cells. Thus, hydrogel-based delivery of IL-10 should improve its utility as a monotherapy and provide a useful adjuvant treatment for approved adoptive cell therapy and checkpoint immunotherapies that are limited by T cell exhaustion.

Tier 1 — Clinical/Translational

PI: Jeremy Meier, MD, PhD, Hematology Fellow

Project Title: Enhancing CAR-T Cell Imaging and Delivery in Solid Tumors

"Chimeric antigen receptor (CAR) T cell therapy has revolutionized the treatment of patients with hematologic malignancies, though its application in solid tumors remains limited, owing in part to challenges in adequate cell trafficking to the tumor microenvironment (TME) and poor persistence once

there. Novel strategies are therefore needed to optimize the effectiveness of CAR T cells in solid cancers. A major limitation in our ability to enhance CAR T activity in these cases stems from our poor understanding of CAR T cell dynamics in vivo. Better delineation of their biodistribution would provide insight on how to maximize their migration to tumors, though progress in this area is currently hindered by our limited capacity to image CAR T cells in patients.

Fluorine-19 (19F) magnetic resonance imaging (MRI) is a novel strategy for in vivo cell tracking that takes advantage of 19F perfluorocarbon probes (PFCs), which are biologically and chemically inert molecules, to label cells for downstream imaging applications. Our preliminary data shows we can successfully label CAR T cells with a novel 19F compound, allowing us to better evaluate CAR T cell fate in vivo. We aim to further characterize 19F labeled CAR T cells and use their imaging potential to screen for novel interventions to increase CAR T cell tumor trafficking, the results from which could be readily translated to CAR T clinical trials. The ability to better image CAR T cells is key to expanding their use in solid tumors and for improving CAR T cell treatment efficacy overall.

Tier 1 — Population Science

PI: Jacob Stein, MD, MPH, Assistant Professor, Medicine

Project Title: UNC AYA Cancer Program: Reach and Influence on Cancer Care Delivery

Abstract

Adolescents and Young Adults (AYAs, defined as age 15-39) are a growing population of patients with cancer, yet have poorer outcomes and care experiences than their younger or older counterparts. They have unique needs based upon their age and developmental stage, but these needs often go unmet by a cancer care delivery system designed for older or younger patients. Given these distinct and diverse needs, many professional organizations and national agencies have recommended AYA-specific programs to help meet this important gap in cancer care. However, little is known about the current landscape of AYA cancer care delivery and how it would be influenced by AYA-specific programs.

We propose a retrospective cohort study of AYAs receiving cancer care at UNC. We aim to describe who they are and what cancer care they are receiving in order to understand the diversity of needs for AYAs at UNC and better tailor our care to meet them. We will explore the clinical and sociodemographic characteristics associated with receiving AYA-specific care as a part of the AYA Cancer Program, enabling a better understanding of who is currently being reached and where to target expansion efforts. Lastly, we will compare survival, cancer treatment, complications, and supportive care outcomes between those who do and do not receive AYA-specific care to offer a preliminary evaluation of how AYA programs can influence tangible cancer-related care delivery outcomes.

Tier 1 — Population Science

PI: Ahrang Jung, PhD, RN, Assistant Professor, Adult Health Nursing, UNC Greensboro

Project Title: Psychosocial Burdens and Supportive Care Needs in Adults with Newly Diagnosed Acute Myeloid Leukemia and Their Caregivers

Abstract

Acute myeloid leukemia (AML) is an aggressive hematologic malignancy with a poor prognosis and high risk of mortality. Over two thirds of patients with AML will not survive five years. Older adults with AML and individuals with significant comorbidities historically experienced especially poor outcomes because

they could not tolerate intensive AML treatments. In the past 4 years, oral venetoclax with infusional hypomethylating agents has substantially improved treatment efficacy for older adults. This new treatment has also changed the treatment setting to outpatient, and now requires patients with AML and their family caregivers to manage their symptoms and treatment within the home environment. However, there are no evidence-based guidelines or interventions for patients and family caregivers to support treatment adherence and quality of life (QOL) of patients taking novel therapies of AML. To address this gap, we propose a cross-sectional mixed-method study to elucidate patients' and family caregivers' experiences with novel home-based therapies and identify factors contributing to their QOL. To accomplish this goal, we will use semi-structured interviews and validated symptom measures with both patients (n=20) and their family caregivers (n=20). This proposed study will provide the necessary preliminary data to support future grant applications focused on the design and evaluation of future psychosocial interventions for patients with AML and their family caregivers.

Tier 2— Basic Science

PIs: Daniel Dominguez, PhD, Assistant Professor of Pharmacology, and Maria Aleman, PhD, Assistant Professor, Pharmacology

Project Title: Hyperactivation of an RNA binding protein by a cancer-associated mutation

Abstract
Altered RNA regulation is a recurrent feature in cancer. Here, we characterize a mutation in RNA binding protein, PCBP1, that occurs in colorectal cancers. Surprisingly, we found that this point mutation in PCBP1 hyperactivates the protein by triggering multimerization. Multimerized PCBP1 bound RNA with heightened affinity compared to its wildtype counterpart and displayed increased RNA regulation. Extensive biochemical and biophysical characterization described how the mutation triggered a conformational change in PCBP1 to support the hyperactive state. Here we seek to test how mutant PCBP1 elicits pro-tumorigenic phenotypes. Specifically, we will test how mutant PCBP1 post-transcriptionally regulates the WNT signaling pathway, a commonly altered pathway in colon cancer. We propose to generate a mouse model of PCBP1 to test tumor initiation and progression. Together this work will uncover post-transcriptional mechanisms driving colorectal cancer. Furthermore, we propose that hyperactivation of RBPs by mutations that induce multimeric states is a recurrent paradigm in cancer.

Tier 2— Basic Science

PI: Michael Emmanuele, PhD, Associate Professor, Pharmacology

Project Title: Targeting the ubiquitin system in triple negative breast cancer

Abstract
Triple-negative breast cancer (TNBC) is an aggressive and highly metastatic subtype of disease. Precision medicines use to treat other breast cancer subtypes are ineffective in the management of TNBC. There is a significant clinical need to identify new targets and consider new strategies to address the disease. Like phosphorylation, ubiquitination controls proliferation of normal and neoplastic cells. Many cell cycle regulators, oncoproteins and tumor suppressors are controlled by ubiquitin (e.g., p53, c-Myc, cyclins, etc.). Despite its importance in cancer phenotypes, little is known about the role of ubiquitin signaling in the etiology and treatment of TNBC. Deubiquitinases (DUBs) are catalytic proteases that shape protein landscapes by antagonizing protein degradation, and several are implicated in cancer. Highlighting their therapeutic potential, selective DUB inhibitors have emerged for several enzymes, and

the first DUB inhibitors recently entered clinical trials. USP7 is an established cancer DUB that is recurrently overexpressed in breast cancer. Despite an established role in controlling p53, USP7 controls myriad pathways the control cell proliferation and survival independent of p53 status. Our strong preliminary data indicate that USP7 represents a therapeutic candidate in TNBC. Our goal is to test its role in TNBC and determine its therapeutic potential. We approach this challenge using deep, quantitative proteomics to decode its influence on the TNBC protein landscapes, combined with cell and animal studies to reveal its role in tumor growth and migration. Altogether, these studies could further nominate USP7 as a therapeutic candidate, and provide key preliminary data needed for a future funding.

Tier 2— Basic Science

PI: Kelsey Fisher-Wellman, PhD, Assistant Professor, Physiology, Eastern Carolina University

Project Title: Targeting mitochondria in acute myeloid leukemia

Abstract
Unlike solid tumors which can at times be removed through surgical intervention, cancers of the blood are primarily treated using various small-molecule drugs referred to as chemotherapeutics. The main drawback of these drugs is that in addition to killing cancer cells, they often also promote the death of non-cancerous cells, including red blood cells and cells of the immune system. In addition to these "off-target" toxic effects, cancer cells often develop resistance to chemotherapy, preventing the surviving cancer cells from being treated with the currently available chemotherapeutics. This is particularly true for patients suffering from acute myeloid leukemia (AML), where disease relapse occurs at an alarming rate. The current project is designed to specifically address the clinical problem of AML relapse by testing a new class of therapeutic compounds that we recently discovered to possess a unique ability to kill both chemosensitive and chemoresistant AML cells. Importantly, these novel drug-candidates eradicate cancer cells while sparing normal healthy cells.

The development of new cancer therapies typically requires scientific discoveries that pinpoint unique characteristics of cancer cells. Over the past several years, we and others have shown that the metabolism of the cancer cell is fundamentally different from non-cancerous cells. A large collection of these cancer vs non-cancer metabolic differences originate within the energy-producing organelle of the cell referred to as the mitochondrion. We now understand that the mitochondrial alterations present in AML are critically important for defending AML against cell death. In the present project, we aim to dismantle AML's defense system using a family of small molecules that, in the laboratory setting, specifically interfere with mitochondria inside AML cells and cause them to die. Importantly, similar effects are observed even in AML cells that are resistant to "traditional" chemotherapies. Therefore, in this project, we plan to test the efficacy of targeting mitochondria in AML as a new treatment strategy against relapsed/refractory AML. Because this strategy is not expected to impact the body's healthy cells, a major benefit of this approach is that these new "mitochondrial-targeted" drugs will lead to the death of only cancer cells and therefore eliminate much of the secondary toxicity typically observed with chemotherapy.

Although the development of new drugs for the clinic does take considerable time, a few of our identified candidate drugs are already FDA approved, albeit for non-AML indications. Therefore, results from our work could lead to either the rapid repurposing of existing compounds for use in AML or the development of new drugs. Ultimately, our long-term goals are to afford patients diagnosed with blood cancer with better, more targeted therapies that have considerably fewer harmful side effects.

Tier 2 — Clinical/Translational

PIs: Jessica Thaxton, PhD, Associate Professor, Cell Biology, and Jonathan Serody, MD, Professor, Medicine

Project Title: Remodeling Th/Tc17 CAR-T Cell Biology to Enhance Power and Persistence in Solid Cancers

Abstract
Barriers to CAR-T cell efficacy against solid cancers include poor persistence, immunosuppression, and exhaustion in the tumor microenvironment (TME). CAR-T cell pools enriched with central memory (Tcm) and stem cell memory (Tscm) products exhibit robust durability in solid tumors, but reasons for persistence are poorly understood. Recently, we demonstrated that STING agonist 2'3'-cGAMP enhanced generation of Tcm and Tscm pools in vivo in Th/Tc17 Neu.CAR-T cells infused into Neu-expressing NT2 breast cancers, enabling a 25% cure rate in immune-competent hosts. A cell-intrinsic reason for CAR-T cell inefficacy in solid tumors is protein stress signaling in the endoplasmic reticulum (ER) that underlies oxidative cell damage, metabolic deterioration, differentiation, and death. Chronic perturbations in environmental homeostasis cause prolonged errors in protein folding that engage chronic stress signaling in the ER. We established that the solid TME induces prolonged protein stress in T cells infiltrating solid tumors followed by oxidative cell damage and aging, that enables cancer progression. Now, our preliminary data indicate that 2'3'-cGAMP acts to mediate Tscm and Tcm development through engagement of a novel antioxidant sensor, Peroxiredoxin-1 (Prdx1), that reprograms Th/Tc17 CAR-T cell metabolic efficacy in tumors, enabling CAR-T cells to bypass induction of the cell response to stress, enhancing persistence and antitumor immunity in solid tumors. This research program will combine the expertise of Serody (Th/Tc17 CAR-T cells) and Thaxton (ER Stress and Immunometabolism) to test that 2'3'-cGAMP stimulates in vivo durability of Th/Th17 CAR-T cells through engagement of Prdx1, enabling attenuation of the cell response to stress.

Tier 2 — Clinical/Translational

PI: Ashwin Somasundaram, MD, Assistant Professor, Medicine

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

Abstract
In the past decade, the ability to further characterize pancreatic cancer (PC) by molecular subtypes has provided not only prognostic value but predictive value. Our exceptional team at UNC including Dr. Jen Jen Yeh, Dr. Naim Rashid, and Dr. Margaret Gulley have established the CLIA-certified and validated PurIST (Purity Independent Subtyping of Tumors) classification tool to retrospectively identify basal subtype PC. Basal PC have worse survival compared to the more common "classical" subtype of PC and is less responsive to standard platinum-based chemotherapy. However, basal PC has improved outcomes with gemcitabine-based chemotherapy (GnP). Our preclinical data has identified differential expression of EGFR (Epidermal Growth Factor Receptor) in basal tumors which may explain why patients with basal tumors per PurIST respond better to EGFR inhibitor therapies such as erlotinib (E). The combination of gemcitabine with erlotinib has been evaluated in two clinical trials where patients with basal subtype tumors had significant improvement in overall survival (OS) 11.67 months with the combination of gemcitabine with anti-EGFR therapy compared to 3 months with gemcitabine alone and no difference between arms was noted in patients with classical subtype tumors. Our central hypothesis is that low-dose EGFR inhibitors in combination with bi-weekly GnP for patients with basal tumors based

on our PurIST classification in an extended phase Ib clinical trial will be safe, tolerable, and efficacious. This project will establish the proper dosing and tolerability of erlotinib in combination with GnP and justify a larger phase III randomized, biomarker-driven, study.

Tier 2 — Population Science

PI: Alison Lazard, PhD, Associate Professor, Hussman School of Journalism and Media

Project Title: Using social media designs to reduce cancer misinformation

Abstract
Cancer misinformation on social media exerts a heavy burden on cancer patients, survivors, and care networks where unproven and potentially harmful treatments and cures are widely shared. Cancer misinformation about false treatments and cures can negatively impact psycho-social health by causing emotional distress. Cancer misinformation also compromises physical health outcomes if individuals use advice to deviate from evidence-based clinical care. Misinformation represents upwards of 30-77% of cancer information on social media and is often shared by cancer care networks as misguided altruism. We need to redirect community action to intervene to reduce cancer misinformation. Most U.S. adults, including Latinos/as, own or access a smartphone (85%), are online constantly or multiple times daily (85%), and use visual-based social media (81%). Social media strategies to reduce cancer misinformation and the negative impacts for those in cancer treatment and survivorship are needed. One promising solution is to use social media designs (prompts with messages) to encourage prosocial intervening (e.g., flagging harmful content). We will develop, translate to Spanish, and evaluate social media designs (prompts with messages) to encourage prosocial intervening and reduce sharing misinformation in a realistic, fully controlled social media platform. We will establish the feasibility, infrastructure, and protocols to test social media designs (prompts with messages) in English and Spanish among cancer care networks. This work will provide evidence for multilingual communication strategies to reduce cancer misinformation with community action on social media.