Bankhead-Coley Cancer Research Program Proposal Review

Grant# 22B01

10074

PI: Vadaparampil, Susan

Institution: H. Lee Moffitt Cancer Center and Research Institute

HPV MISTICS: HPV Multilevel Intervention Strategies Targeting Immunization in Community Settings

Human Papillomavirus (HPV) vaccination is a safe and effective strategy to reduce incidence and mortality from multiple cancers that affect men and women. Men and women in Florida suffer from HPV-related cancers at higher rates than the national average; yet, only 56.0% of 13-17-year-old adolescents are up to date with HPV vaccination, representing a significant missed opportunity to reduce HPV-related cancer incidence and mortality. Although evidence-based strategies (e.g., communication training, reminders, education) to improve HPV vaccine rates exist, Florida physicians' use of these strategies is low. Utilizing an innovative partnership with the Health Choice Network (HCN), the proposed project features a Hybrid Type 1 effectiveness-implementation, stepped-wedge randomized controlled trial design to assess the effectiveness of HPV Multilevel Interventions Strategies Targeting Immunization in Community Settings (HPV MISTICS) in federally qualified health centers (FQHCs). Informed by the Competing Demands Model, the HPV MISTICS intervention leverages evidence-based interventions at the provider-, parent-, and system-levels. The provider-level intervention is a 1-hour online training session, led by a Physician Educator, on how to effectively utilize the Announcement Approach (i.e., presumptive recommendation) to recommend adolescent vaccinations. The parent-level intervention includes a low literacy pre-visit HPV vaccine notification postcard. The system-level intervention involves training a Vaccine Champion in each FQHC on utilization of Florida's statewide immunization registry (Florida SHOTS) to monitor clinic and provider level HPV initiation and completion rates, generate and share individual monthly reports for providers on their patient panel's HPV vaccination uptake, and implement a reminder/recall to notify patients about subsequent HPV vaccine doses. Additionally, we will use mixed methods to assess implementation outcomes guided by the RE-AIM QuEST framework to explore whether implementation outcomes are equitable across FQHCs and the patients served and to identify implementation barriers and facilitators. The primary outcomes are HPV vaccine series initiation and completion rates for patients ages 11-17 in participating FQHCs. The proposed aims are to: 1) test whether the multilevel HPV MISTICS intervention increases HPV vaccine series initiation rates and completion rates among adolescents ages 11-17; 2) explore potential covariates of intervention effect; and 3) explore equity of implementation outcomes (reach, adoption, implementation, maintenance/sustainability) and identify implementation barriers and facilitators. By partnering with HCN, study findings can be disseminated and implemented in FQHCs nationwide, demonstrating the high potential to improve public health.

Grant# 22B02

10051

PI: Lau, Eric

Institution: H. Lee Moffitt Cancer Center

The trouble with testosterone: delineating how androgen drives melanoma invasiveness and metastasis via fucosylation-regulated cellular adhesion

Sex is an emerging prognostic indicator for melanoma—men exhibit poorer outcomes than women. Melanoma incidence and mortality rates are higher for men, with ~50% more new cases and twice the lethality in men than women in the US in 2020. Consistently, the male hormone androgen/testosterone has been reported to increase melanoma progression. We find that androgen induces melanoma proliferation and invasiveness. Thus, the therapeutic inhibition of androgen signaling represents an attractive new treatment strategy for melanoma, given the availability of clinical androgen receptor (AR) antagonists for prostate cancer. However, we lack mechanistic insights and biomarkers that are essential for informing which pathological and therapeutic contexts—which melanoma patients—would benefit from inhibition of AR. Thus, studies elucidating key androgen- and AR-regulated mechanisms that drive melanoma pathogenesis are urgently needed.We discovered a significant mechanistic connection between sex and melanoma fucosylation (modification of proteins with the sugar L-fucose). Melanomas in men exhibit lower global fucosylation levels than in women. Fucosylation can promote or suppress tumors—divergent functions that are dictated by 13 rate-limiting, tumor-promoting or tumor-suppressing fucosyltransferases (FUTs) that conjugate L-fucose onto different glycans on target proteins. Consistent with reduced melanoma fucosylation in men, androgen reduces fucosylation in melanoma cells by suppressing expression of the global fucosylation substrate regulator FUK. However, androgen simultaneously increases a tumorigenic form of fucosylation synthesized by a pro-tumorigenic FUT, FUT4. Androgen activates transcription of FUT4 by AR, and FUT4 overexpression potently drives melanoma invasiveness. Phosphoproteomic profiling highlighted adherens junction (AJ) signaling as the most significantly AR-FUT4-regulated pathway, which likely underlies AR-induced invasiveness in melanoma. Our data indicate that AR induces tumorigenic fucosylation to drive melanoma pathogenesis by increasing invasiveness. The ability to block invasiveness using clinically approved AR antagonists is expected to significantly change treatment paradigms for melanoma. Here, we propose to determine how AR-FUT4-AJ regulates melanoma invasiveness and potential correlations of AR-FUT4-AJ signatures with clinical parameters. We will test our HYPOTHESIS that AR-FUT4 regulates AJ signaling to drive melanoma invasiveness and metastasis in 2 Specific Aims (SAs):SA1. DETERMINE HOW AR-FUT4 REGULATES AJ SIGNALING & INVASIVENESS IN MELANOMA. We will determine how:(i) FUT4 regulates key AJ components (proteomic/biochemical analyses, map signaling)(ii) FUT4-AJ signaling regulates melanoma biology/invasiveness. (in vitro cell-based assays using cells manipulated for FUT4/AJ component(s)) SA2. DELINEATE CONTRIBUTIONS OF AR-FUT4-AJ TO MELANOMA METASTASIS IN VIVO & ASSESS CORRELATIONS OF AR-FUT4-AJ SIGNATURE WITH CLINICAL PARAMETERS. We aim to determine how AR-FUT4-AJ drives melanoma metastasis in vivo and which melanoma patients might benefit from AR antagonists. We will:(i) delineate effects of AR antagonists & contribution of FUT4-AJ to AR-induced metastasis (mouse models using AR antagonists and melanoma cells as in SA1.ii).(ii) study correlations of AR-FUT4-AJ components with metastasis/clinical parameters in humans. (patient tissue & large expression dataset analyses)

Grant# 22B03

10004

PI: Conejo-Garcia, Jose

Institution: Moffitt Cancer Center

Heterogeneity of metastatic small cell lung cancer; implications for the design of effective immunotherapies

Small cell lung cancer (SCLC) is an aggressive malignancy, but it is characterized by many mutations, which provides a rationale for designing immunotherapeutic interventions. Accordingly, combined immune checkpoint blockade (a form of immunotherapy) has recently elicited objective responses, while immunotherapy after chemotherapy increased overall survival. Nevertheless, SCLC remains a major clinical challenge due to presentation at advanced, metastatic stages, and rapid progression. Aggressive malignant progression and spreading has also limited a clear understanding of the progression of the human disease, and the design of more effective treatments as a result. To address these barriers and move the field forward, Moffitt Cancer Center has established a Rapid Tissue Donation (RTD) program that provides timely access to the entire repertoire of metastatic lesions in terminal patients, who generously donate their tissues for this research. Using this unique resource, we plan to characterize the barriers that impair the effectiveness of immunotherapies, with a focus on the heterogeneity of the disease, and novel interventions to overcome these hurdles. Based on our expertise on tumor immunology (Dr. Conejo-Garcia) and clinical immunotherapy (Dr. Perez), as well as access to unique clinical specimens provided through an effective Rapid Tissue Donation program, we postulate that the effectiveness of immunotherapies against small cell lung cancer is thwarted by heterogeneous immunogenicity across different tumor masses, along with immune cell sequestration and metabolic restrictions at tumor beds. Based on our preliminary results, our central hypothesis is that pleural effusions and tumor-infiltrating immune cells that “snap” chunks of tumor cell membranes (“trogocytic” T cells) will provide a source of effector lymphocytes able to target multiple tumor masses; provided that metabolically-driven cell stress pathways are co-targeted. We propose the following Specific Aims: Aim 1. Define intra-and inter-tumor heterogeneity in the immunogenicity of human small cell lung cancer. Aim 2. Elucidate the trajectory of differentiation of tumor-reactive T cells in small cell lung cancer. Aim 3. Design cellular therapies that target heterogenous metastatic disease. Insight derived from these approaches will, first, define the role and heterogeneity of neoantigens and tumor microenvironmental immune cells in small cell lung cancer; a poorly characterized disease, due in part to its aggressiveness. Most importantly, we will provide the field with a mechanistic rationale for more effective immunotherapies that target the diversity of this human disease.

Grant# 22B04

10151

PI: Smalley, Inna

Institution: H. Lee Moffitt Cancer Center and Research Institute, Inc.

Defining and targeting the immune-suppressive metabolic microenvironment of leptomeningeal melanoma metastases

Florida is second in the nation for the highest rates of melanoma cases, the deadliest form of skin cancer. Despite the remarkable progress in the development of targeted and immune therapies against advanced melanoma, most patients ultimately relapse. One of the most serious complications of advanced melanoma is spread of cancer cells to the leptomeninges and their infiltration into the cerebrospinal fluid (CSF), collectively known as leptomeningeal melanoma metastasis (LMM). The leptomeninges and CSF are uniquely immunosuppressive and are emerging as a more frequent, common site of melanoma progression. Clinically, patients with LMM have very short survival times regardless of treatment (typically 8-10 weeks) and no FDA-approved therapies exist for these patients. There is an urgent clinical need to better understand the biology of LMM and to identify LMM-specific therapeutic targets. The goal of this proposal is to improve our understanding of leptomeningeal melanoma metastasis and identify therapeutic vulnerabilities for this disease. In our preliminary single-cell transcriptomic analysis of LMM, and melanoma metastases from the brain and skin, we have identified major differences in potential metabolic programs of the tumor specific to the leptomeninges. Furthermore, we found significantly higher proportions of infiltrating macrophages with a transcriptional signature associated with an alternative, pro-tumorigenic phenotype. Traditionally, metabolism has been viewed as a collection of catabolic and anabolic pathways that generate energy and biosynthetic precursors required for growth and survival. However, emerging evidence suggest broader roles for metabolic processes in controlling other aspects of physiology, including immune cell functions. We believe that the metabolic adaptations melanoma cells undertake to survive in the unique microenvironment of the leptomeninges alter the metabolism of infiltrating immune cells, leading to an increase in immune tolerance and tumor survival. We will define the main metabolic adaptations that drive the immune suppressive environment in LMM-resident macrophages and identify the ideal axis for therapeutic intervention that inhibits LMM progression. We will utilize a ground-breaking integrated single-cell metabolomics and RNAseq approaches, paired with tracing of metabolic flow to examine how LMM-specific changes in melanoma metabolism impact the alterations of key metabolic pathways of macrophages. We will then utilize our innovative cell culture and animal models of LMM to test if targeting LMM-specific metabolic adaptations in melanoma cells or the LMM-driven metabolic remodeling of macrophages will attenuate the immune- suppressive environment and inhibit LMM progression. These data will lay the groundwork the development of clinically relevant therapeutic approaches for LMM.

Grant# 22B05

10001

PI: Kissil, Joseph

Institution: Moffitt Cancer Center

Establishing the functional differences between variant oncogenic KRAS alleles and identification of allele-selective inhibitors

The RAS proteins (HRAS, KRAS and NRAS) are small G-proteins that function as master regulators of signaling pathways conveying stimuli from the extracellular environment that impact cellular behaviors such as proliferation, death and differentiation. They are the most frequently mutated oncogenes in

cancer, with mutations found in approximately a third of all cancers. The KRAS gene is the most frequently mutated with mutations found in pancreatic carcinomas (>90%), lung adenocarcinomas (>30%) and colorectal tumors (>40%). In these tumors, the activity of the oncogenic KRAS protein is required for the proliferation and/or survival of the tumor cells and thus represents a high-value target for therapeutic development. Extensive efforts to target the RAS proteins, have been ongoing but have proven to be challenging due to multiple reasons stemming from the biology of the RAS proteins, complexity of downstream effector pathways and upstream regulatory networks. An important lesson learned from research over the past several years is that not all mutant RAS alleles are created equal. Previously, it was thought that the major mutations in the RAS proteins, those altering amino acids G12, G13 or Q61, all function in a similar manner to impair GTPase activity, thus resulting in the balance of cellular RAS being in the GTP-bound, active (ON) state. However, several studies clearly demonstrate that different RAS alleles lead to different functional outcomes and sensitivities. These findings underscore the potential for identifying specific biology associated with each of the different oncogenic RAS alleles and exploiting this therapeutically, as demonstrated by recent successes with development of small-molecules directly targeting the oncogenic KRAS-G12C variant. Our long-term goals are to understand the functions of the different RAS alleles and identify specific vulnerabilities that can be exploited for therapeutic gain. Towards this goal we will focus on mutations in KRAS using a newly developed allelic series of isogenic lung adenocarcinoma (LuAD) cells. This series, which consists of a panel of isogenic LuAD cells that differ only in the status of KRAS at codon G12 or G13, was created using CRIPSR-based editing, and will allow us to carefully characterize and compare the effects of these mutations at a cellular level, permitting elucidation of the differential signaling events downstream of the oncogenic KRAS variants using novel transcriptomic and proteomic approaches. Importantly, we will employ additional innovations we have already shown to dramatically improve discovery of small molecules that are selective against oncogenic KRAS. These include the use of a 3D spheroid-based screening format, which we have previously shown to expose new vulnerabilities that would not have been identified in traditional 2D format assays. Finally, we will employ cutting-edge approaches for target deconvolution by functionalizing the identified screening hits with diazirine and alkyne moieties for in-cell UV crosslinking and click chemistry for target pull-down respectively, where cross-linked targets will be identified by mass spectrometry. These efforts will greatly facilitate target identification and determining the mechanism of action of the identified hits.

Grant# 22B06

10050

PI: Manley, Brandon

Institution: H. Lee Moffitt Cancer Center and Research Institute, Inc.

Establishing the role of aberrant splice variants as a clinical biomarker in clear cell renal cell carcinoma

Our proposal is an innovative study that seeks to evaluate the role of specific molecular splicing events that frequently occur with the most common type of kidney cancer, clear cell renal cell carcinoma (ccRCC). We are investigating the role of this novel splice variants as biomarkers in patients with locally advanced disease who have a high chance of recurrence. Splice variants have demonstrated clinical applicability in treating patients in several solid malignancies, including lung cancer, prostate and brain cancer but their role or impact among ccRCC remains unknown. Building on the strong scientific rationale of our previous studies, this proposal will produce several impactful short-term results. Our

candidate list of 6 aberrant splice variants are ideal biomarkers for ccRCC given their specificity, high frequency of occurrence in ccRCC tumors. Additionally, these molecular events happen in the same location of selected candidate genes in all ccRCC patients making them an excellent target for assay development. The results of our study will define the ability of these aberrant splice variants to be detected in the plasma of patients with ccRCC and how their presence or absence after surgery may predict detect early recurrence of disease. Successful results of our study would overcome critical steps to the first blood-based clinical biomarker test for kidney cancer patients. We have designed our proposal in such a fashion that successful detection of the splice variants in the plasma of patients using digital polymerase chain reaction (dPCR) will allow for rapid integration to the clinic. This technology is highly sensitive, economically practical for large scale testing and already employed for clinical use to identify alterations in genes such as epidermal growth factor receptor (EGFR) gene and the B-Raf proto-oncogene (BRAF).Furthermore, several results drawn from our proposed research could have long-term impacts on the field of kidney cancer in its goal to improve patient survival. Upon demonstrating the clinical role of these biomarkers and development of an accurate testing assays, future studies can further refine its ability to guide systemic treatment strategies. Our previously published data shows a possible correlation with poor response to immunotherapy in those patients whose tumor have a splice variant of EGFR. Development of personalized therapy strategy is important since there remains little comparative data from currently approved drugs, a wide diversity of drug mechanisms and an ongoing emergence of combination treatments that lack clarity on which patients should receive a specific treatment. Additionally, by demonstrating the clinical applications of splice variants we can expand research to better understand the biological repercussions of these alterations and how we may further therapeutically exploit them. Lastly, by demonstrating impact in locally advanced ccRCC patients we will be well positioned to investigate the possible role of aberrant splice variants in the early detection or screening for ccRCC, especially in high-risk populations. These outcomes synergize with the ultimate goal of extending the life expectancy and eliminating the disease-related deaths of Florida residents and others with kidney cancer.

Grant# 22B07

10064

PI: Luca, Vincent

Institution: Moffitt Cancer Center

Structure-guided engineering of LAG3 immunomodualtory function

Over the past several years, immunotherapy has emerged as a highly effective treatment for cancer. In contrast to chemotherapy, which kills cancer cells with toxic chemicals, immunotherapy teaches a patient's immune system to eradicate tumors. As current immunotherapy treatments are only successful in~ 30% of cases, scientists are actively searching for ways to create new classes of immunotherapy drugs. We are using two different methods to guide the development of next-generation immunotherapies. Our first strategy is to use a high-resolution imaging technique called x-ray crystallography to "see" how the receptor LAG3 sends signals that suppress immune cell function. By visualizing LAG3 molecules on the atomic scale, our goal is to obtain molecular blueprints that inform the design of more effective drugs. For our second strategy, we will harness these blueprints to engineer decoy proteins that can block incoming signals to the LAG3 receptor. These decoys will then be used to block LAG3 from shutting down the immune response. Initially, the LAG3 decoys will be used to re-

activate T cells in a laboratory setting. However, if these tests are successful, our long-term goal is to proceed to clinical trials in melanoma patients.

Grant# 22B08

10002

PI: Thompson, E Aubrey

Institution: Mayo Clinic

Spatial analysis of the immune landscape of stage 4 triple negative breast cancer

Locally advanced or metastatic triple negative breast cancer (Stage 4 TNBC) is a devastating disease with near 100% mortality. Standard of care for previously untreated Stage 4 TNBC involves immuno-therapy with an antibody against the immune checkpoint inhibitor PD-L1 (atezolizumab or pembrolizumab) plus chemotherapy (usually a taxane). A subset of patients who receive such therapy have clinical benefit, defined as stable disease or tumor regression for at least six months. However, about half of the patients receive no benefit and progress rapidly with lethal consequences. The clinical challenges are 1) to understand why some patients benefit, 2) to Identify those patients who are unlikely to benefit, and 3) to develop alternative therapeutic strategies for such patients. We are motivated by the core concept that a rational approach to immuno-therapy of TNBC requires a detailed understanding of the numbers, types, activities, and location of immune cells within the tumor mass. In pursuit of this objective, we have played a major role in the development of NanoString GeoMx digital spatial profiling (DSP) technology. Our laboratory was one of four academic sites, world-wide, that was selected for beta testing of this technology; and we have, to date, processed >1600 samples, mostly breast cancer. We are confident that we have more experience with this technology than any laboratory outside of NanoString.DSP technology involves multi-plex digital spatial quantification of antibody binding to a single 5-micron formalin-fixed, paraffin-embedded (FFPE) section. The dynamic range is at least 5 logs, and the output is digital: we count the number of antibody molecules bounds and, from those counts, infer spatially defined abundance of 81 key immune and other target proteins. GeoMx technology has recently been developed for measuring spatially defined abundance of 18,000 transcripts (WTA: whole transcriptome analysis) in a single 5-micron FFPE section. This technology therefore enables us, for the first time, to measure spatial multiplex protein and transcript profiles and to define relationships between therapeutic outcome and these features in TNBC.We have recently completed DSP analysis of 319 early stage TNBC tumors. Four highly interactive key features were identified as associated with good prognosis: 1) intraepithelial antigen-presenting activity (APC); 2) intraepithelial T cell activation status (TCA); 3) intraepithelial PD-L1 abundance; and 4) intraepithelial IDO1 abundance. We will carry out DSP analysis to test the hypothesis that these intraepithelial makers, APC, TCA, PD-L1 and IDO1 alone or in combination, are associated with clinical benefit in Stage 4 TNBC patients who receive anti-PD-L1 immunotherapy plus chemotherapy. A secondary analysis will involve whole transcriptome spatial analysis to identify targetable features that may be exploited to improve clinical benefit in patients who do not benefit from PD-L1 plus chemotherapy. Our goals are to provide a comprehensive spatial analysis of the immune landscape of Stage 4 TNBC, identify features that may guide therapeutic decision making in patients who present with Stage 4 disease, and identify potential therapeutic targets that can be exploited for management of advanced TNBC patients who fail standard of care anti-PD-L1 therapy.

Grant# 22B09

10024

PI: Minond, Dmitriy

Institution: NSU

Spliceosomal modulation for regulation of melanoma immunogenicity

As estimated by the National Cancer Institute (NIH/NCI), there are more than 900,000 people living with melanoma in the US. In Florida, approximately 700 people die from melanoma every year and >7000 new cases are diagnosed every year (http://www.flhealthcharts.com), which makes melanoma treatment one of the top research priorities in Florida. Despite recent advances in melanoma drug discovery, the average overall survival of patients with late-stage metastatic melanoma is ~3 years. Instances of complete response are very rare; therefore, more life-prolonging therapies are needed. This suggests a need for new approaches and targets for melanoma drug discovery. The objective of this proposal is to determine the role of spliceosomal proteins hnRNPH1 and H2 (H1 and H2, 96% homology) in melanoma immunogenicity, which could lead to the novel approaches to therapy, which is one of the research priorities set forth by Florida Biomedical Research Advisory Council. Our preliminary findings suggest that small molecule modulation of spliceosome can lead to the increase of melanoma cell immune signaling, which can be beneficial to the patients. We are proposing the following specific aims: (1) Determine role of H1/H2 in melanoma immunogenicity in vitro; (2) Determine role of H1/H2 in melanoma immunogenicity in vivo; and (3) Determine in vivo efficacy of spliceosomal modulation in combination with immunotherapy. Our team is uniquely positioned to successfully execute the Aims of this study. Drs. Venkatesan and Velayutham (NSU) bring their expertise in animal and molecular studies. Overall, these proof-of-principle studies will provide evidence of role of H1/H2 in melanoma immunogenicity and will form a basis for further studies to assess its potential for therapy.

Grant# 22B10

10061

PI: Licht, Jonathan

Institution: The University of Florida

Mitochondrial modulators of multiple myeloma growth and therapy resistance

Background: Multiple myeloma (MM), an incurable blood malignancy of antibody producing plasma cells, is of great importance to Florida due to its association with aging and much higher incidence among black populations. Despite introduction of new therapies, most patients relapse due to drug resistance. MM arises in part from acquired chromosomal anomalies that yield high-level expression of cancer-causing genes. Most therapies used for MM target malignant cells based upon their plasma cell nature and do not target specific mutations or translocations. Chromosomal translocation t(4;14), found in 15% of MM, leads to overexpression of the histone methyltransferase NSD2 which drives an oncogenic gene expression program associated with poorer prognosis. Identification of genes required for MM cell growth, including high-risk subtypes like t(4;14), is a new way to begin development of

novel therapies Preliminary data and premise: Through a gene disruption fitness screen in NSD2 high and low MM cells, we identified genes whose loss is more detrimental to NSD2 high cells. Among these was the gene encoding adenylate kinase 2 (AK2). The Broad dependency map database indicates that only a few cell types require AK2 for growth, including MM with t(4;14). AK2, localized in the mitochondrial intermembrane space, catalyzes the reversible reaction ADP + ADP = AMP + ATP. High antibody production MM puts cells under endoplasmic reticulum (ER) stress, increasing the need for energy (ATP) to fold proteins. Our initial data suggests that AK2 is indeed required to resolve ER stress in MM cells, possibly due to its ability to generate ATP. Rapid AK2 depletion activates unfolded protein response (UPR) cell death signaling. Analysis of Multiple Myeloma Research Foundation and our own data indicate that AK2 overexpression is linked to MM resistance to proteasome inhibitors, therapies that kill MM by generating ER stress. We hypothesize that MM growth depends on mitochondria energy production and AK2 to prevent ER stress, representing a therapeutic vulnerability. Therefore, our aims will be: Aim 1: Define the molecular basis of the dependence of NSD2 high MM on AK2. We will determine how NSD2-mediated changes in gene expression affects mitochondrial metabolism and dependence on AK2. NSD2 overexpression may prevent metabolic adaptation to AK2 depletion. We will determine how altered apoptotic UPR signaling in NSD2-high MM cells affects susceptibility to AK2 loss. Aim 2: Characterize the role of AK2 in MM in vitro and in vivo. We will determine the effect of AK2 depletion on proliferation, migration, invasion, and apoptosis in cell culture and mouse models. The effect of AK2 disruption on mitochondrial function, cell metabolism and gene expression in MM will be ascertained. Aim 3: Investigate the role of AK2 and other mitochondrial constituents in MM fitness and therapy response within the bone marrow microenvironment. The effect of AK2 knockdown and overexpression on the ability of MM cells to form spheroids in a 3D model resembling bone marrow will be investigated. The 3D culture will be used to determine how AK2 affects sensitivity of MM cells to therapeutic agents. The role of AK2 in modulating MM growth and response to PIs will be assessed in vivo using the MOPC315.BM syngeneic mouse model. Functional screens of nuclear-encoded mitochondrial genes will be performed in vivo to elucidate the role of mitochondria in MM progression and response to PI therapy.

Grant# 22B11

10089

PI: Jin, Lingtao

Institution: University of Florida

The Role of Immune Microenvironment in Small Cell Lung Cancer

Lung cancer is the leading cause of cancer-related death in the US and worldwide, among both men and women. Lung cancer claims more lives than do colon, prostate, ovarian and breast cancers combined each year. Small cell lung cancer (SCLC) that accounts for around 15% of lung cancer cases is the most aggressive subtype of lung cancer with a five-year survival rate of less than 5%. The results of numerous clinical trials have been disappointing and to date no approved targeted-therapy for small cell lung cancer is available. As a result, treatment options for small cell lung cancer have not had the same progress as non-small cell lung cancer (NSCLC). Immunotherapy such as anti-PD1 and anti-PD-L1 antibodies that boost immune system to eliminate cancer cells has demonstrated unprecedented clinical activity in several difficult-to-treat cancers including non-small cell lung cancer but has only showed modest efficacy in small cell lung cancer. The immunotherapy drug durvalumab and

atezolizumab (anti-PD-L1 antibody) have recently received FDA approval as a first line therapy. Compared with chemotherapy alone, however, adding durvalumab or atezolizumab only extends patient median overall survival by two months. Such modest efficacy of immunotherapy drugs observed in small cell lung cancer highlights the unmet need for more effective combination therapy approaches. The mechanistic basis for this impaired anti-tumor immunity in small cell lung cancer remains unknown but mounting evidence suggests that the tumor microenvironment plays a key role in determining the efficacy of immunotherapy.Dendritic cells (DCs) are professional antigen-presenting cells that play a key role in orchestrating immune responses against tumor development. However, various immunosuppressive factors in the tumor microenvironment undermine DC function. Importantly, immune dysfunctional DCs result in uncontrolled tumor progression, indicating that maintaining the immune competence of DC is critical for successful anti-tumor immunity. It has long been suggested that accumulation of lipids in the tumor microenvironment (TME) drive DC dysfunction. The underlying mechanism, however, is unexplored. In this proposal, we hypothesize that lipid-laden DCs in the tumor microenvironment are induced by tumor-derived exosomes (TDEs), small vesicles released by tumor cells. We uncover that TDE-derived long-chain fatty acids critically contribute to lipid accumulation and consequently dysfunction of DCs in SCLC. DCs uptake TDEs with large amount of fatty acids that activates peroxisome proliferator activated receptor a (PPARa) signaling, a master regulator involved in lipid metabolism. The activation of PPARa in DCs further leads to aberrant lipid accumulation, which culminates in the induction of immunosuppressive enzyme arginase 1 (Arg1) and consequently dysfunction in DCs. Importantly, inhibition of PPARa effectively correct the immune dysfunction of DCs, and enhanced anti-tumor efficacy of immunotherapies in SCLC. Collectively, our findings indicate that TDEs, as fatty acid carriers, adversely affect DCs function, and that targeting PPARa could be a novel therapeutic strategy for small cell lung cancer.

Grant# 22B12

10081

PI: Barrientos, Antonio

Institution: University of Miami Miller School of Medicine

Targeting mitochondrial protein synthesis to combat blood malignancies

Targeted signaling inhibitors for hematologic malignancies may lead to limited clinical efficacy due to the outgrowth of subpopulations with alternative pathways independent of the drug target. Relapse/refractory disease that results from treatment with targeted signaling inhibitors is a major hurdle in obtaining curative responses. Interestingly, work over the past decade or more has shown that chronic myelogenous leukemia (CML) stem cells (CD34+CD38-) are resistant to targeted signaling inhibitors, such as the BCR-ABL kinase class of inhibitors, often a problematic source of resistance leading to minimal residual disease. Recent studies have shown that some forms of lymphoma and leukemia cells have an energy metabolism highly dependent on mitochondrial oxidative phosphorylation. Tigecycline, a US FDA-approved antibiotic, inhibits the synthesis of mitochondrion-encoded proteins due to the similarity of bacterial and mitochondrial ribosomes, leading to selective lethality in hematologic malignancies reliant on enhanced oxidative phosphorylation. Indeed, it was established that CML stem cells are reliant on upregulated oxidative phosphorylation and a combination of imatinib and tigecycline could eradicate therapy-resistant CML, both in vitro and in animal models. The main goal of this proposal is to determine the mechanism by which elatol inhibits mitochondrial translation and its usefulness to target mitoribosomes as a therapeutic strategy against

several types of leukemia. We hypothesize that the dependence of leukemia cells on OXPHOS makes them especially vulnerable to inhibition of mitochondrial protein synthesis. We will develop two specific aims to test this hypothesis:Aim 1. Characterize the general metabolomics flux and specifically mitochondrial energy metabolism, gene expression, and OXPHOS system organization in an array of leukemia and lymphoma cell lines in comparison with healthy bone marrow and blood cell linesAim 2. Determine the sensitivity of oxidative and glycolytic leukemia and lymphoma cells to pharmacological interference of mitochondrial protein synthesis by using classical and new drugs (elatol) that act as mitoribosome inhibitors, alone or in combination with imatinib or other currently used therapeutic agents. We anticipate that these studies will shed light on the energetic metabolism of leukemia cells and will determine the suitability of genetic and pharmacological interventions that target mitochondrial translation to eliminate leukemia cells selectively.

Grant# 22B13

10018

PI: Schatz, Jonathan

Institution: University of Miami Miller School of Medicine

Inhibition of the Cell-Cycle Kinase GAK, a Novel Therapeutic Target in Diffuse Large B-Cell Lymphoma

New treatments are needed for patients with diffuse large B-cell lymphoma (DLBCL), an aggressive blood cancer diagnosed in nearly 3,000 Floridians annually. We performed a specialized screen for new drug targets and discovered that inhibiting the enzyme cyclin-G associated kinase (GAK) is a promising strategy for attacking DLBCL tumors while sparing normal blood cells. GAK carries out alignment of the machinery that pulls chromosomes apart during cell division. We found that exposing DLBCL cells to a GAK inhibitor halted their cell division and promoted programmed cell death (apoptosis), showing their particular dependence on this process to maintain malignant behavior. B-cells are among the most rapidly dividing of all cells in the body, and DLBCL tumors derived from them grow aggressively in patients but also are especially dependent on proteins that carry out cell division. Non-malignant blood cells from healthy donors were not substantially affected by GAK inhibition, demonstrating tumor specificity of effects, which should lead to a safe therapeutic window for use in human patients. Other cell-division proteins have been assessed as drug targets in the past in DLBCL and other cancers, and drugs against them remain under development. Our data reveal GAK as a new target with several advantages, including its unique activities during cell division, its highly drug-targetable kinase enzymatic activity, and preliminarily a specific biomarker for tumors likely to be sensitive to its inhibition (dysfunction of a specific tumor suppressor whose loss is a very common driver of malignancy including DLBCL). These findings provide a strong opportunity to develop novel approaches for the treatment of DLBCL and, in the longer term, potentially additional malignancies as well. This application seeks support for these efforts fueled by two specific aims that address the crucial next steps in this process: First, we will define in detail the molecular consequences of GAK inhibition in DLBCL experimental systems (Aim 1). These studies will reveal the reasons DLBCL tumors are particularly dependent on GAK for survival and the DLBCL disease subtypes in which GAK inhibition is likely to be most effective. This aim includes also treatment studies in highly accurate animal DLBCL models to preliminarily define the therapeutic window for GAK inhibition in mammalian organisms. Second, we will leverage our multidisciplinary team of researchers to design and synthesize new GAK-specific inhibitors for further development (Aim 2). Currently, no GAK inhibitor is suitable for testing in patients due to major pharmacologic issues with

existing tool compounds, which have fueled our studies to date. We already have identified a series of highly promising chemical structures that are predicted by computer modeling to be potent GAK inhibitors and highly suitable for further optimization for eventual use in patients. In particular, we have identified a strong possibility to generate irreversible inhibitors of GAK through permanent (covalent) binding to the target, a strategy that has yielded some of the most potent and effective drugs against cancer and other diseases in the past. This wholly novel target-validation and drug-development effort combines multidisciplinary expertise into an exciting opportunity to bring about better clinical outcomes for cancer patients in Florida.