James & Esther King Biomedical Research Program Proposal Review

Grant# 22K01

20062

PI: Ross, Owen

Institution: Mayo Clinic Florida

Creating a Florida Cerebrovascular Disease Biorepository and Genomics Center

The Global Burden of Disease Study ranked cerebrovascular disease as the second leading cause of death. Stroke is associated with significant morbidity and mortality. Standardizing phenotypic data collection and identifying genetic variants that determine risk for cerebrovascular diseases will offer new insight into the development of these conditions and may provide personalized treatment for these diseases. We have established the Mayo Clinic Florida Cerebrovascular Diseases Registry (CDR) to build a biorepository of specimens from patients who enter the clinic. We have collected a series of 1000 patients (53% female; mean age, 60.4 years) as of July, 2021), including over 200 from under-represented minority communities. These patients undergo extensive clinical phenotyping. The registry classifies patients by one or more of 23 cerebrovascular conditions as well as concurrent stroke-free healthy control subjects. Eligible conditions include cerebral infarction, cerebral hemorrhage, ruptured and unruptured cerebral aneurysms, and cavernous malformations. Each eligible cerebrovascular condition has strict requirements for evidence of the disease based on imaging, laboratory results, or diagnostic guidelines. Participants undergo a structured intake assessment of demographic variables, stroke risk factors, stroke symptoms, neurological impairment, mental status, and functional status pre- and post-symptom onset. A detailed family history is obtained and affected and unaffected family members are encouraged to participate. We have performed genetic screening in a small number of cases from the CDR to rule out known genetic causes and identify novel disease determinants. The goal of the proposed studies will be to expand the registry, not only based on Mayo Clinic Florida samples, but also to other academic medical centers in Florida. We will propose DNA sequencing in collected samples to build a genomic sequencing-bioinformatics pipeline for studies of cerebrovascular disease in Florida. In addition, over 10% of the samples collected thus far are from minorities including African American and Hispanic populations. We have already started genetic analysis in these populations and propose to prioritize collection and analysis in these cohorts. Mayo Clinic Florida was the first center in Florida to receive the Advanced Certification as a Comprehensive Stroke Center by The Joint Commission. This distinction identifies centers that are focused on providing advanced and complex stroke care. Collaborators Dr. Jose Romano from the University of Miami and Dr. Scott Silliman from the University of Florida will provide additional support for this research infrastructure by aiding in the recruitment of diverse populations with young onset and familial cerebrovascular disease. Overall, we plan on initiating a multi-center biospecimen repository for cerebrovascular disease that will expedite the identification of novel variants implicated in cerebrovascular disease and lead to the development of

prognostic tools, advanced therapies and treatment, and improved techniques for early detection methods.

Grant# 22K02

20030

PI: Vidrine, Jennifer

Institution: Moffitt Cancer Center

Enhancing Long-Term Smoking Abstinence Among Cervical Cancer Survivors

One-third of cervical cancer survivors smoke, and this prevalence of smoking is higher than among any other subgroup of cancer survivors. This is a critically important clinical and public health issue in cancer survivorship given that smoking plays a significant role in causing cervical cancer and that continuing to smoke is associated with increased risk of recurrence, second primary cancers and other diseases. Cervical cancer survivors represent a special population with complex behavioral and environmental risk factors and are likely to require a tailored approach to treatment. National data indicate that within one year of diagnosis, 90% of cervical cancer survivors who smoke attempt to quit, yet only 35.5% of these individuals are successful. The vast majority of smoking cessation interventions for cancer survivors have failed to demonstrate efficacy. Thus, there is a pressing need for efficacious and sustainable interventions with broad dissemination potential. The proposed study has been designed to help fill this need. Our team recently completed an NCI-funded randomized controlled trial which evaluated the efficacy of a theoretically- and empirically-based "Motivation And Problem-Solving" (MAPS) telephone counseling approach to facilitating smoking cessation among cervical cancer survivors. MAPS is a holistic, dynamic behavior change intervention that utilizes a combined motivational interviewing (MI) and social cognitive theory based approach. MAPS is designed for all smokers regardless of their readiness to quit and specifically targets motivation, social cognitive constructs, and other factors of key relevance to cervical cancer survivors through a Wellness Program. Our results indicate that, compared to a quitline treatment control group, MAPS resulted in greater than a 2-fold increase in smoking abstinence at 12 months (i.e., 23.2% vs. 10.3%), coinciding with the end of the treatment period. Unfortunately, this treatment effect was no longer significant at 18 months (i.e., 11.3% vs. 10.4%), which suggests that efficacy dissipated as time from end of MAPS treatment increased. This dissipation of the treatment effect over time does not appear to be driven by dropout as retention was high throughout the study (i.e., 82% at 18 months). Rather, findings support participants' motivation for long-term engagement in treatment and highlight the need for sustained intervention. Our overall goal is to develop and test a novel treatment adjuvant to MAPS that will extend treatment and enhance long-term abstinence from smoking. This adjuvant will build upon participants' relationships with the MAPS counselors. Cervical cancer survivors who currently smoke will be recruited throughout Florida via social media and randomly assigned to one of two treatment groups: 1) Standard Treatment [ST] or 2) Extended MAPS [MAPS-EXT]. ST participants will be connected to the FL tobacco quitline. MAPS-EXT will consist of proactive counseling calls delivered over 12 months plus a novel treatment adjuvant that will extend through 18 months. All participants will receive 12 weeks of combination nicotine replacement therapy (i.e., patch + lozenge) and will be followed for 24 months. The primary aim is to evaluate the efficacy of MAPS-EXT vs. ST in facilitating long-term smoking abstinence. A secondary aim is to compare the magnitude of the mediated effects via common treatment mechanisms on smoking abstinence between the MAPS-EXT and ST treatment groups.

Grant# 22K03

20057

PI: Doonan, Bently

Institution: University of Florida

Novel RNA-Nanoparticle vaccine for treatment of early melanoma recurrence following adjuvant anti-PD-1 antibody therapy

Melanoma is an increasing public health concern in the state of Florida with 7900 new diagnoses annually. The advent of immune checkpoint inhibitors (ICI) has revolutionized the treatment of advanced cutaneous and mucosal melanoma. Based on their profound impact on advanced disease they have also become the standard of care in the adjuvant setting in resected stage III/IV melanoma following surgery. These agents have shown a prolonged recurrence-free survival in these high-risk melanoma patients, however, up to 30% of patients will have disease recurrence within 1 year of treatment. Previous studies have also shown that patients who develop disease recurrence following adjuvant ICI treatment, or while on treatment, have a more aggressive course of disease that does not respond to PD-1 inhibitors and has a poor response to CTLA4 inhibition. Furthermore, patients with mucosal melanoma who had recurrence did not benefit from subsequent ICI therapy at all, highlighting this subtype of melanoma as an area of high unmet clinical need. One reason for the failure of ICI in the post adjuvant setting is the immune suppressive nature of the melanoma tumor microenvironment (TME) and lack of professional antigen presenting cell (APC) activation. These APCs, namely dendritic cells (DC) often remain in an inert state unable to present tumor antigens for immune detection due to lack of innate immune cell triggering and inhibition from myeloid derived suppressor cells (MDSCs). This innate arm of the immune system works to prime and drive the natural immune response to both virally infected cells and against malignancy which then activates the adaptive immune T cell response against the tumor. We have developed a novel (FDA approved) RNA-nanoparticle (RNA-NP) vaccine that simultaneously penetrates and reprograms the TME while inducing a tumor specific adaptive T cell response. This vaccine utilized novel engineering design that layers tumor derived mRNA into a lipid-nanoparticle (NP) “onion-like” package. These NPs enable maximal isolated and stable packaging of mRNA into a fixed volume for easy distribution, cell-penetration, and uncoating which quickly boosts innate and adaptive immune responses. These RNA-NPs localize to the TME and activate multiple innate pathways thereby activating DC and suppressing the function of MDSCs. In this study we propose the use of patient derived RNA-NP vaccine in patients with early recurrence of melanoma who previously received or are currently receiving PD-1 therapy. We propose that through re-priming of the antitumor immune response and alteration of the TME we can improve the efficacy of PD-1 therapy and prevent the need for subsequent lines of therapy which have little current clinical value. If effective this treatment will revolutionize the management of this aggressive subset or melanoma patients and improve overall survival. This study will also gather important information into the mechanisms of early ICI resistance, identify novel biomarkers of innate cell resistance and response to treatment, and provides a cutting edge, personalized immunology approach to melanoma treatment.

Grant# 22K04

20038

PI: Law, Brian K

Institution: University of Florida College of Medicine

Toward IND-Enabling Studies for Novel Cancer Therapeutics that Inhibit the Disulfide Isomerases ERp44, PDIA1, and AGR2

Breast cancer is among the malignancies caused by smoking and remains one of the largest causes of cancer death in American women. The TRAIL protein that is produced by immune cells selectively kills cancer cells, while sparing normal cells, through activation of its receptors Death Receptors 4 and 5 (DR4/5). Anticancer agents that act through the TRAIL-DR4/5 pathway have not yet been approved for breast cancer therapy due to limitations in the stability of TRAIL and insufficient levels of DR4/5 in some cancers. Our team has discovered that breaking individual DR5 disulfide bonds stabilizes high-level DR5 production, and fully activates its toxicity toward cancer cells without the need for TRAIL stimulation. This novel mode of DR4/5 activation is triggered by mutation of individual Cysteine residues involved in one of the 7 extracellular disulfide bonds of DR4 or 5, or using new pharmacological agents developed by our team termed Disulfide bond Disrupting Agents (DDAs). We hold one issued patent and several pending patent applications on DDA composition of matter, and the use of DDAs as anticancer agents. Recent work shows that DDAs are inhibitors of the Protein Disulfide Isomerase (PDI) enzymes ERp44, PDIA1, and AGR2 that control the disulfide bonding of select subsets of proteins. Of note, no active site ERp44 or AGR2 inhibitors have been reported, highlighting the novelty of DDAs. The mechanisms of DDA anticancer action have been investigated and their activity against breast tumors in experimental models is well established. We have developed several third-generation DDAs that exhibit high potency and selectivity. However, studies have not been completed to determine which of these compounds is the most promising candidate for advancement to IND-enabling work that will include Good Manufacturing Practice (GMP) compound production, followed by detailed metabolism and toxicology studies. The goal of the work proposed here is to determine which of the third-generation DDA candidates has superior stability, in vivo anticancer efficacy, and maximal target engagement in tumor cells. In parallel, the optimal method of DDA administration and formulation will be determined. The results of these studies will focus our follow-up IND enabling efforts on the DDA candidate with the greatest potential for success in clinical trials. As such, the results of this project will increase the potential for commercializing DDAs through acquisition of conventional basic research grants from NIH and DoD, and through small business funding (e.g., SBIR/STTR) from NIH, DoD, or FDOH.

Grant# 22K05

20026

PI: Liao, Daiqing

Institution: University of Florida

Development of first-in-class HDAC3-selective degraders for breast cancer therapy

Breast cancer (BC) is the leading cause of cancer burden for women, diagnosed in over 1 million worldwide each year. BC affects one in 20 women globally and as many as one in eight in Western countries. Although more and more patients with BC have survived of the disease, over 450,000 patients die of this disease annually. About one third of invasive BCs progress to recurrent or metastatic disease, and ~90% of BC deaths are due to metastatic cancer. There are several major breast cancer subtypes:

estrogen receptor-a (ER)-positive (ER+), HER2-enriched and triple-negative (TNBC). All BC subtypes can progress to distant metastases. Metastatic BC is currently incurable. The short median survival of 3 years for patients with metastatic BC has not significantly changed in over 20 years. Therefore, more effective treatments are urgently needed to combat BC.A class of cellular enzymes known as histone deacetylases (HDACs) are associated with promoting BC progression and treatment resistance in BC. HDAC inhibitors (HDACi) such as entinostat and tucidinostat have shown clinical anticancer efficacy in combination with the aromatase inhibitor exemestane. This indicates that targeting HDACs are promising new therapy for treating BC. Notably, HDAC3, a specific HDAC isozyme, also has function independent of its enzymatic activity, which appears to be important for its oncogenic property. Conventional HDACi cannot block this function of HDAC3, suggesting that existing HDACi could not adequately ablate HDAC3's oncogenic function. Therefore, novel strategies are needed to effectively inhibit HDAC3 in cancer cells. Proteolysis targeting chimeras (PROTACs) targeting various oncogenes have shown promising anticancer effects. Importantly, improved target selectivity can be achieved by converting a conventional non-selective inhibitor to a PROTAC. We have designed and synthesized novel PROTACs that degrade HDAC3 with a high potency and selectivity. These novel compounds potently impaired BC cell viability at a very low drug concentration. In this application, we propose to optimize and validate the HDAC3 PROTACs for potency and selectivity in degrading HDAC3. We will also determine (1) the mechanism of action of these novel HDAC3 PROTACs, (2) their in vivo drug properties, (3) their safety profiles, and (4) their anticancer and anti-metastatic efficacy in preclinical animal studies. The outcome of this project will provide critical proof-of-concept evidence for potentially translating the first-in-class HDAC3 PROTACs into the clinic for treating patients with advanced BC.In summary, our goal in this project is to develop novel treatment regimens that are highly effective, broadly applicable, less toxic, and improve survival. By the end of the three-year funding period, we expect to identify highly effective HDAC3 degraders for preclinical development. Our team has the experiences in advancing drug candidates to clinical trials. We anticipate that clinical trials may start immediately after this project is completed. Ultimately, the novel compounds may enter the clinic for treating patients with all BC subtypes.

Grant# 22K06

20040

PI: Nagathihalli, Nagaraj

Institution: University of Miami

Targeting CREB to Improve Response to Immunotherapy in Pancreatic Cancer

Tobacco smoking is a significant risk factor for pancreatic and other cancers. Nearly one in five adult Floridians smoke regularly, making this a significant public health concern. Pancreatic cancer is currently the third leading cause of cancer-related death in the United States and the mortality rates from this disease are expected to surpass breast, prostate, and colorectal cancer by 2030. Tobacco induces a systemic inflammatory response resulting in fibrosis of the pancreatic parenchyma providing a milieu for the progression of cancer. Understanding the mechanism by which smoking increases the risk of pancreatic cancer or any cancer is still being unraveled. We recently identified that in vivo tobacco smoking could lead to increased tumor growth and activation of a protein called Cyclic AMP Response Element-Binding 1 (CREB), a downstream mediator of the oncoprotein Kras, which is a key driver in pancreatic cancer. CREB is activated in both smokers and non-smokers with pancreatic cancer, however, its activation is significantly higher in smokers. We now present preliminary data in murine models of pancreatic cancer that reveals the critical importance of CREB in promoting smoking-

dependent pancreatic inflammation, fibrosis, and immunosuppression through the recruitment of myeloid and regulatory T cells. We further show that activated CREB promotes transcriptional upregulation and secretion of an immunosuppressive myeloid chemoattractant leukemia inhibitory factor (LIF), as a possible mediator of tumor myeloid cross talk. Based on these findings, we hypothesize that smoking suppresses the anti-tumor immune response, and targeting CREB can overcome the inflammatory-immunosuppressive milieu to improve the response to immunomodulatory drugs. This proposal's overall objective is to delineate the mechanisms by which CREB modulates the immune tumor microenvironment in the tobacco-associated and spontaneous pancreatic cancer model and explore CREB inhibition as a potential target for the treatment. In aim 1, we identify CREB-regulated transcriptional mechanism and use selective CREB inhibition to deplete immunosuppressive subsets, further enabling the adaptive immune response to engage and eliminate tumor epithelial cells in tobacco smoking models. In aim 2, we investigate whether inhibition of CREB-LIF signaling enhances T cell recruitment and activity in tobacco-associated pancreatic cancer and whether a subset of these recruited T cells develops an exhausted phenotype. We will further determine the immunologic changes using high throughput technologies in genetically engineered mice harboring a knockout of the CREB gene. Finally, in aim 3, we evaluate if CREB inhibition with conventional immunotherapeutic approaches will improve survival and augment the immune response in mice. We will incorporate anti-immune treatment additively to CREB inhibition to activate ant-tumor immune responses. Impact: Our proposed studies elucidate the molecular signatures which will lead to the discovery of innovative strategies that can counteract the harmful carcinogenic effects of tobacco smoking. This project will also generate significant new insights into how smoking alters the inflammatory pathways in pancreatic cancer to promote disease progression. Additionally, the results of this study will broadly be applicable to the myriad of other malignancies where tobacco smoking is a risk factor. Therefore, this proposal is innovative, novel, and significant.

Grant# 22K07

20041

PI: Vazquez-Padron, Roberto

Institution: University of Miami

The Impact of Smoking in the Venous Cellular Ecosystem and its Consequences for Arteriovenous Fistula Maturation in CKD Patients

The number of passive and active smokers living with end-stage renal disease (ESRD) whowill eventually need a functional vascular access to receive hemodialysis and extend their lives is increasing. The arteriovenous fistula (AVF) created by anastomosing an arm vein to a nearby artery is the preferred vascular access because it poses fewer complications than central venous catheter and arteriovenous grafts. However, AVF failure due to venous stenosis (narrowing) remains a significant morbidity for dialysis patients and significantly contributes to hemodialysis costs. The conventional methods to re-open failed fistulas, such as balloon angioplasty and stenting, can temporally extend the life of the access but do no prevent the progressive dysfunction even after decades of medical optimization. This highlights a clear need for in-depth research initiatives that apply state-of-the-art omics technology to clinically relevant tissues to dissect the cellular and molecular mechanisms leading to failure and to discover how smoking deteriorates vascular health compromising the function of the AVF and shortening the life of ESRD patients. Herein, we will create the first single cell atlas of the human vein of smokers and non-smoker patients that could explain the susceptibility to failure after AVF creation. We

apply state-of-the-art genomic technology to our unique tissue biobank that contains more than 800 human veins and AVF. Our overreaching goal is to identify the changes in the venous "cell ecosystem" that ultimately lead to AVF failure. Our fundamental hypothesis is that smoking changes the cell ecosystem in the pre-access veins that is responsible for vascular wall repair and remodeling and that a disequilibrium in those cells prior anastomosis increases the risk for focal stenosis in the outflow vein of the AVF. In this proposal, we will integrate emerging single-cell omics technologies to definitively characterize the dynamics of cell subpopulations in the pre-access vein of active smokers and non-smoker patients. Outcomes from this novel proposal will help identify and spatially characterize known and unknown cell subpopulations before and after AVF creation that will generate new insights into the mechanisms leading to smoking-related AVF failure.

Grant# 22K08

20006

PI: Li, Ji

Institution: University of South Florida

Sirtuin 1 and Cardiovascular Impairment by Cigarette Smoking

Cigarette smoking is a major preventable cause of morbidity and mortality worldwide. It is estimated that >5 million people die from tobacco smoke-related illnesses each year. Smoking is a major independent risk factor for systemic injury, including atherosclerotic vascular disease, hypertension, and stroke. While the association between chronic smoking and cardiovascular disease is recognized, the underlying mechanisms are incompletely understood. There is emerging evidence that a longevity protein sirtuin 1 (SIRT1) can ameliorate systemic injury caused by cigarette smoking. Moreover, our group revealed that SIRT1 agonists play a critical role in cardioprotection against age-related cardiovascular injury through modulating metabolic homeostasis and inflammatory response. Thus, it is hypothesized that pharmacological SIRT1 agonists can ameliorate smoking-induced cardiovascular insults of hypertension patients via maintaining the metabolic and redox homeostasis. Two aims are proposed to test the hypothesis: Aim 1, to determine the mechanisms by which cigarette smoking cause down-regulation of cardiac SIRT1 signaling. Aim 2, to define the capability of small-molecule SIRT1 agonists to ameliorate cardiovascular damage by cigarette smoking under pressure overload conditions. The inducible cardiomyocytes specific SIRT1 knockout (icSIRT1-/-) mice and wild-type littermates (SIRT1f/f) will be exposed to whole-body mainstream cigarette smoke using a SCIREQ smoking system. Age-matched, air-exposed mice will serve as nonsmoking controls. The pharmacological SIRT1 agonism will characterize the critical role of SIRT1 in ameliorating systemic injury caused by cigarette smoking exposure. In this manner, we will advance our understanding of the mechanisms underlying the cardiac SIRT1 signaling cascade in response to smoking-induced pathological stress. This grant seeks the potential to discover new therapeutic strategies to rescue cardiovascular impairment caused by cigarette smoking exposure. PUBLIC HEALTH RELEVANCE: The toxicological constituents of cigarette smoke, including nicotine, carbon monoxide, particulates, oxidants, and heavy metals, indicate the potential to cause systemic injury. Moreover, smoking-induced cardiovascular disease is one major leading to systemic injury in human health. This grant aims to understand how impaired systemic signaling causes a higher incidence of cardiovascular insult in the hypertension population and can discover new therapeutic strategies to limit systemic injury by cigarette smoking in these patients.

Grant# 22K09

20025

PI: Mohapatra, Subhra

Institution: University of South Florida

Mechanism of neurotropism by coronaviruses

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), simply called CoV2, which causes coronavirus infectious disease (COVID-19) has rapidly become a global super pandemic. In persons with comorbid conditions such as cardiovascular diseases, cancer, diabetes, obesity, or significant smoking history, CoV2 infection causes pneumonia leading to acute respiratory distress syndrome often leading to death. Also, cancer patients, especially lung cancer patients infected with CoV2 exhibit severe symptoms and a high mortality rate. In addition to respiratory symptoms, over one-third of the CoV2-infected patients show diverse neurological manifestations, including symptoms of stroke, encephalopathy, and loss of smell or taste. Though vaccines are available against COVID-19, the virus continues to mutate to generate highly transmissible and more virulent strains making vaccine efficacy uncertain. Also, about 30% of the population are unwilling to take vaccines or use face masks, which has led to the intense search for new approaches that can significantly decrease the severity and CoV2 neurotropism. Herein, we propose an out-of-the-box intervention strategy that comprises an anti-COVID nasal spray, which is expected to work can work in non-mask user and unvaccinated segment, in addition to breakthrough infections in the vaccinated segment of our population. We have made a serendipitous discovery that CoV2 spike protein molecularly binds to anti-diabetic drug pioglitazone (PG), which decreases brain inflammation in traumatic brain injury. However, PG has low solubility and it does not get into the brain easily. On the other hand, a PG metabolite, lerglitazone (LG), has higher solubility and better ability (versus PG) to cross the blood-brain and was found to dock to COV2 spike trimer and spike-ACE2 interface comprising of Arg403 of integrin-binding RGD motif involved in virus adhesion to and entry into the host cells suggesting that LG could inhibit virus infection. Preliminary studies suggest that CoV2 infects the lung epithelial, glial, and endothelial cells and neurovascular pericytes, and brain cells in mice. Also, LG shows a significant reduction in viral infection by CoV2 and its variants. Further, nano formulated LG given as nasal spray shows more robust antiviral and anti-inflammatory effects in the mouse coronavirus (MHV) model that exhibits neurotropism. These results have led to the central hypothesis that CoV2 migrates from nasal epithelium to the brain causing neuropathology, and that an intranasal LG nanoformulation can effectively reduce CoV2 viral load and attenuate the CoV2-induced neuroinflammation and demyelination, and treat neuroCOVID. To test this hypothesis, it is planned to evaluate the basis of neurotropism of CoV2 and examine oligodendrocyte myelination of axons (aim #1) and determine the efficacy of intranasal LG nanoformulation in reducing CoV2 induced damage to the central nervous system (aim #2). It is expected that the proposed highly innovative studies would demonstrate the efficacy of this neurotropism-directed approach to treating CoV2 infection, which renders the smokers more vulnerable to severe COVID-19 and even death.