Biomedical Research Advisory Council

Bankhead-Coley Cancer Research Program

James and Esther King Biomedical Research Program

Live Like Bella Pediatric Cancer Research Initiative

2019-2020 Annual Report

Ron DeSantis
Governor

Scott A. Rivkees, MD
State Surgeon General for Health

2019-2020 Annual Report - Table of Contents

BIOMEDICAL RESEARCH PROGRAM INTRODUCTION AND OVERVIEW

Since 2001, the Florida Legislature has recognized the need to support vital research conducted in both academic and private institutions throughout the state through the William G. “Bill” Bankhead Jr. and David Coley Cancer Research (Bankhead-Coley) Program (section 381.922, Florida Statutes) and the James and Esther King Biomedical Research (King) Program (section 215.5602, Florida Statutes). In fiscal year (FY) 2019-2020, this funding continued to improve the health of Florida’s families, expanded the research infrastructure of the state, and advanced efforts to bring external research funding to the state. Total funding, in the amount of $16,451,500, was awarded to Bankhead-Coley and King grantees. This funding resulted in 15 Bankhead-Coley and 11 King new research grants.

In 2018, the Legislature appropriated $3 million in research funding as part of the Live Like Bella Pediatric Cancer Research Initiative (Bella). In the first year, five grants were awarded. During FY 2019-2020, nine new grants were awarded to universities and cancer research centers across the state, to support researchers in their efforts to improve prevention, diagnosis, treatment, and to develop cures for childhood cancer.

Research grants are issued based on a competitive peer-review process. Awards are based on scientific merit, as determined by independent peer review involving experts located outside Florida who are free from conflicts of interest. Researchers at any university or established research institute in the state are eligible to apply for state funding. Per statutory requirements, a FY 2019-2020 progress report is to be submitted that includes the following information:

- A list of recipients of program grants or fellowships. For each research project supported by grants or fellowships awarded under the program, the report must include:

  (1) A summary of the research project and results or expected results of the research;
  (2) The status of the research project, including whether it has concluded or the estimated date of completion;
  (3) The amount of the grant or fellowship awarded and the estimated or actual cost of the research project;
  (4) A list of principal investigators under the research project;
  (5) The title, citation, and summary of findings of a publication in a peer-reviewed journal resulting from the research;
  (6) The source and amount of any federal, state, or local government grants or donations or private grants or donations generated as a result of the research project;
  (7) The status of a patent, if any, generated from the research project and an economic analysis of the impact of the resulting patent; and
  (8) A list of postsecondary educational institutions involved in the research project, a description of each postsecondary educational institution’s involvement in the research project, and the number of students receiving training or performing research under the research project.

- The state ranking and total amount of biomedical research funding currently flowing into the state from the National Institutes of Health (NIH).

- Progress toward programmatic goals, particularly in the prevention, diagnosis, treatment, and cure of diseases related to tobacco use, including cancer, cardiovascular disease, stroke, and pulmonary disease.

- Recommendations to further the mission of the programs.

WILLIAM G. "BILL" BANKHEAD, JR., AND DAVID COLEY CANCER RESEARCH PROGRAM

The Bankhead-Coley Cancer Research Program advances progress toward cures for cancer. Cancer is the second leading cause of death for Floridians, with heart disease being number one. Florida continues to have the second highest cancer burden in the nation. Funding through the Bankhead-Coley program significantly improves cancer research and treatment in the state by:

- Attracting new research talent and grant-producing researchers.

- Funding proposals that demonstrate the greatest ability to attract federal research grants.

- Encouraging the development of bioinformatics to allow researchers to exchange information.

- Facilitating technical collaboration, business development, and support for intellectual property related to research.

- Aiding multi-disciplinary research through greater participation in clinical trials networks and reducing the disparate impact of cancer on certain groups.

THE LIVE LIKE BELLA PEDIATRIC CANCER RESEARCH INITIATIVE

The purpose of the Live Like Bella Pediatric Cancer Research Initiative is to advance progress toward curing pediatric cancer through grants awarded through a peer-reviewed, competitive process. The Initiative will provide grants for research to further the search for cures for pediatric cancer, by pursuing the following goals:

- Significantly expand pediatric cancer research capacity in Florida.

- Improve both research and treatment through greater pediatric enrollment in clinical trials networks.

- Reduce the impact of pediatric cancer on disparate groups.

THE JAMES AND ESTHER KING
BIOMEDICAL RESEARCH PROGRAM

The purpose of the James and Esther King Biomedical Research Program is to advance cures in tobacco-related diseases. The King program funds research initiatives that seek new insights and innovative solutions in the prevention, diagnosis, treatment, and cure of Floridians afflicted by tobacco-related diseases including cardiovascular disease, stroke, lung disease, and tobacco-related cancers, the leading causes of death in Florida and nationally.

The long-term goals of the program are to:

- Improve the health of Floridians by researching better prevention, diagnoses, treatments, and cures for cancer, cardiovascular disease, stroke, and pulmonary disease.

- Expand the foundation of biomedical knowledge relating to the prevention, diagnosis, treatment, and cure of diseases related to tobacco use, including cancer, cardiovascular disease, stroke, and pulmonary disease.

- Improve the quality of the state's academic health centers by bringing the advances of biomedical research into the training of physicians and other health care providers.

- Increase the state's per capita funding for research by undertaking new initiatives in public health and biomedical research that will attract additional funding from outside the state.

- Stimulate economic activity in the state in areas related to biomedical research, such as the research and production of pharmaceuticals, biotechnology, and medical devices.

BIOMEDICAL RESEARCH GRANT ADVISORY BOARD OVERVIEW AND MEMBERSHIP

The Biomedical Research Advisory Council (section 215.5602(4), Florida Statutes) advises the State Surgeon General regarding the direction and scope of the biomedical research program. The responsibilities of the council include, but are not limited to:

(a) Providing advice on program priorities and emphases.
(b) Providing advice on the overall program budget.
(c) Participating in periodic program evaluation.
(d) Assisting in the development of guidelines to ensure fairness, neutrality, and adherence to the principles of merit and quality in the conduct of the program.
(e) Assisting in the development of appropriate linkages to nonacademic entities, such as voluntary organizations, health care delivery institutions, industries, government agencies, and public officials.
(f) Developing criteria and standards for the award of research grants.
(g) Developing guidelines relating to solicitation, review, and award of research grants and fellowships, to ensure an impartial, high-quality peer review system.
(h) Reviewing reports of peer review panels and making recommendations for research grants and fellowships.
(i) Developing and providing oversight regarding mechanisms for the dissemination of research results.
(j) The council shall select, by majority vote, six members of the council who must combine with seven members of the Florida Cancer Control and Research Advisory Council to form a joint committee to develop performance measures, a rating system, a rating standard, and an application form for the Cancer Center of Excellence Award created in Section 381.925, Florida Statutes.

The names and positions of each Biomedical Research Grant Advisory Council Member, as of June 2019, are listed below. There is currently one vacancy. (Biographical statements or curriculum vitae available upon request):

Daniel Armstrong, PhD (Chair), Senior Associate Dean for Child Health; Director, Mailman Center for Child Development; Professor and Executive Vice Chair, Department of Pediatrics University of Miami Miller School of Medicine; Seat: American Cancer Society

Richard Nowakowski, PhD (Vice-Chair), Professor and Department Chair of Biomedical Sciences at Florida State University College of Medicine; Seat: Governor

Charles Evans Wood, PhD, Professor and Chair, Department of Physiology and Functional Genomics, University of Florida; Seat: American Heart Association

Allison Eng-Perez, Principal, Deloitte and Touche, LLP; Seat: Governor

David A. Decker, MD, FACP, Professor and Attending Physician, Orlando Veterans Administration Medical Center and University of Central Florida; Seat: Governor

Richard Houghten, PhD, President and CEO, Torrey Pines Institute for Molecular Studies; Seat: Senate

Tushar Patel, MB, ChB, Dean of Research, Mayo Clinic; Seat: Senate

Conor Lynch, PhD, Associate Member, Moffitt Cancer Center; Seat: House of Representatives

Mary P. Martinasek, PhD, MPH, Associate Professor and Assistant Dean of College of Natural and Health Science, University of Tampa; Seat: American Lung Association

Vacant Seat: House of Representatives

Strategic Goals

In 2014, the Biomedical Research Advisory Council (BRAC) created a strategic plan for Florida's biomedical research funding to specify defined objectives to be accomplished in specific time frames. The strategic plan focuses on the health impact of research and making Florida a destination for cancer care and research. This strategic plan also demonstrates the Florida Department of Health's (Department) commitment to transparency in communicating program priorities, defines the BRAC's substantive areas of focus, specifies time frames for evaluating success, and guides funding opportunities issued by the Department. The BRAC recommended that the following strategic goals be included in the funding opportunity announcement.

- Prevention and Treatment
    - Conduct research with a focus on prevention and improved treatment or care delivery that contributes to decreased deaths due to lung cancer by 15 percent, breast cancer by 15 percent, prostate cancer by 20 percent, colon cancer by 25 percent, and melanoma by 15 percent within 10 years.
    - Develop innovative basic and clinical research studies focused on lower incidence of high mortality/high morbidity cancers (e.g., sarcomas, pancreatic tumors, central nervous system (CNS) tumors, myeloma, leukemia/myelodysplastic syndrome) that result in significant improvement in survival/quality of survival in adults and children in at least two of these cancers.
    - Enhance understanding of the relationship between obesity, healthy weight, and cancer.
    - Improve screening accuracy, detection of high-risk subgroups, and/or improved implementation of cancer screening programs that result in a 20 percent increase in early detection of cancer or preventable cancer within 10 years.
- Technology Transfer Feasibility (TTF)
    - Establish at least five Investigational New Drug applications or Investigational Device Exemptions based on Florida investigator drug discovery, biologic, or other therapeutics that result in at least two multi-center collaborative clinical trials within 10 years.

- Design research protocols that lead to academic-industry development of five new biotechnology products/companies that subsequently obtain incremental commercial funding (beyond Florida funding) within 10 years.

- Health Disparities

  - Develop research that contributes to reductions in deaths due to lung cancer by 30 percent, breast cancer by 30 percent, prostate cancer by 30 percent, colon cancer by 30 percent, and melanoma by 30 percent resulting from health disparities due to race, ethnicity, or income within 10 years.

- Tobacco Use

  - Reduce tobacco use in children and adolescents to less than 4 percent and adults to less than 15 percent within 10 years.

- Treatment Related Morbidities

  - Expand upon research that improves scientific understanding of causes and subsequent impact of cancer/cancer treatment-related morbidities in other systems (e.g., cardiovascular, pulmonary, endocrine, lymphatic, CNS, reproductive, developmental).

- Investigational New Drug (IND) or Investigational Device Exemption (IDE)

  - Supports the development of IND and IDE applications to the United States Food and Drug Administration (FDA) as part of an application for marketing. The intent is to support promising new drug discovery and commercialization of new drugs.

FY 2019-2020 funding cycle awards were made to support the following research priorities for Bankhead-Coley, King, and Bella initiative grants:

27 Awards – Prevention and Treatment: (12 Bankhead-Coley, 8 King, and 7 Bella) Research with a focus on prevention and improved treatment or care delivery that contributes to a reduction in deaths in at least one of the following types of cancers: pediatric, lung, breast, prostate, colon, or melanoma.

No Award –TTF: The goals of the TTF grant mechanism are to stimulate technology transfer activities for promising research discoveries that could lead to innovations in the prevention, diagnosis, treatment, and/or cure of cancer and strengthen a project's economic feasibility and commercialization prospects.

2 Awards – Health Disparities: (2 Bankhead-Coley but 1 was relinquished because of receiving federal research grant funds) Research that contributes to reductions in deaths due to the cancers listed above resulting from health disparities due to race, ethnicity, or income.

No Award – Tobacco Use: Reduction of tobacco use in children, adolescents, and adults.

1 Award – Screening: (1 Bella) Improve screening accuracy, detection of high-risk subgroups, and/or improved implementation of a cancer screening program that results in an increase in early detection or prevention of at least one of the cancers listed above.

5 Awards – Treatment-Related Morbidities: (2 Bankhead-Coley but 1 was relinquished because of receiving federal research grant funds and 3 King) Expand upon research that improves scientific understanding of causes and subsequent impact of cancer/cancer-treatment related morbidities in other systems (e.g., cardiovascular, pulmonary, endocrine, lymphatic, CNS, reproductive, developmental impairment, graft-versus-host disease).

2 Awards –IND or IDE: (1 Bankhead-Coley and 1 Bella) The goal of this mechanism is to expand upon research that supports the development of IND and IDE applications to FDA as part of an application for marketing. The intent is to support promising new drug discovery and commercialization of new drugs.

Figure 1: Bankhead-Coley Applications and Funded Projects

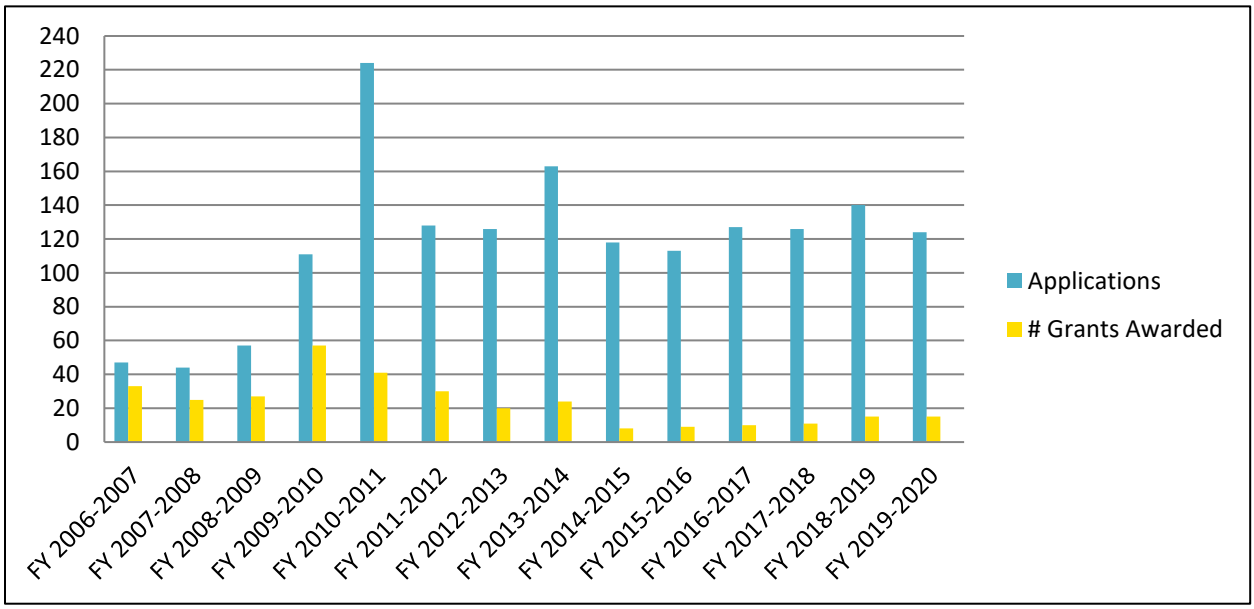


Figure 1: In FY 2019 - 2020, 124 grant applications were submitted in response to the Bankhead-Coley funding opportunity announcement. In FY 2019-2020, 15 cancer-related disease research projects were awarded.

Figure 2: King Applications and Funded Projects

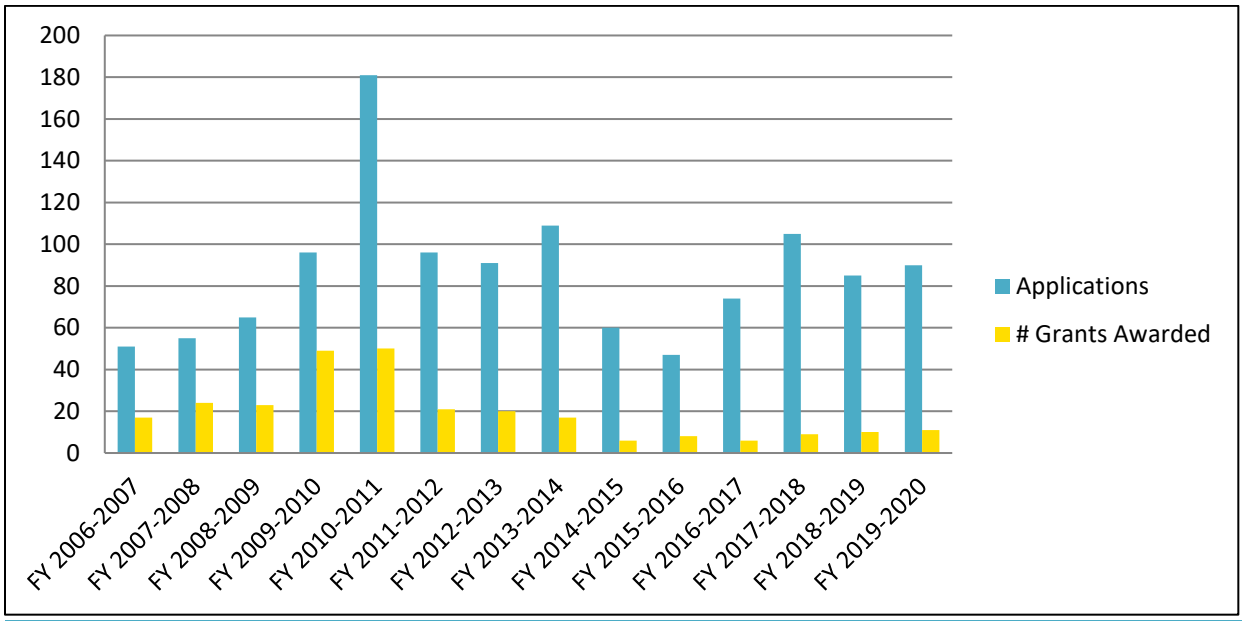


Figure 2: In FY 2019 - 2020, 90 grant applications were submitted in response to the King funding opportunity announcements. During FY 2019 - 2020,11 tobacco-related disease research projects were awarded.

Figure 3: Bella Applications and Funded Projects

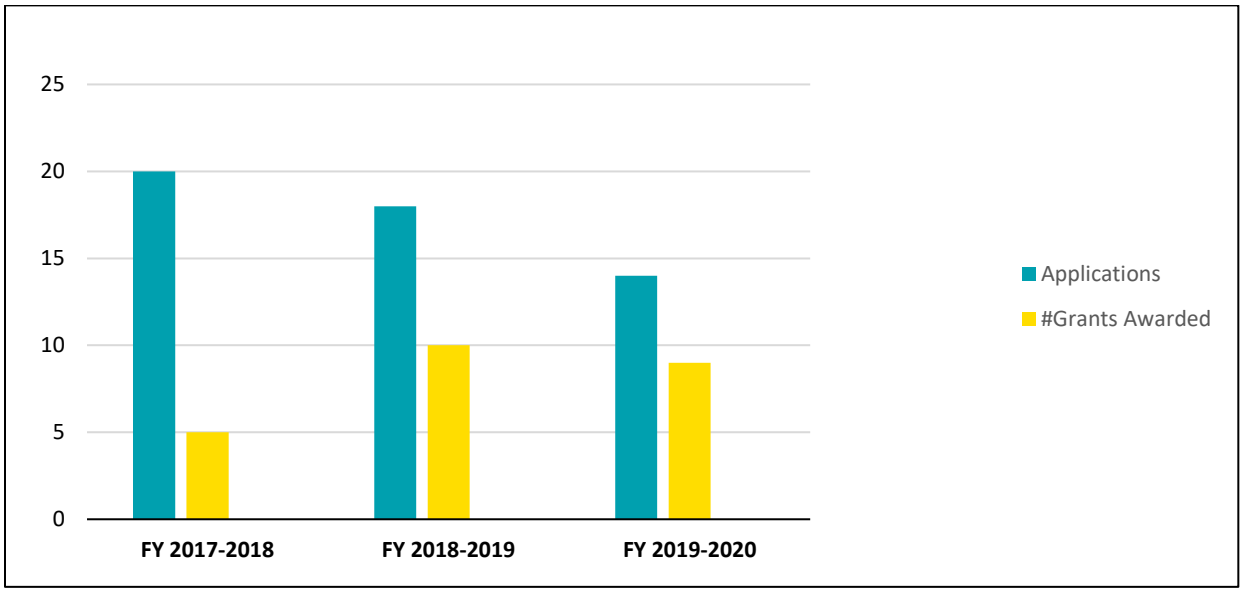


Figure 3: In FY 2019 - 2020, 14 grant applications were submitted in response to the Bella funding opportunity announcement. During FY 2019 - 2020, nine pediatric cancer research projects were awarded. As the program continues to become known, it is anticipated that more grant applications will be submitted.

NIH RESEARCH FUNDING AND STATE RANKING

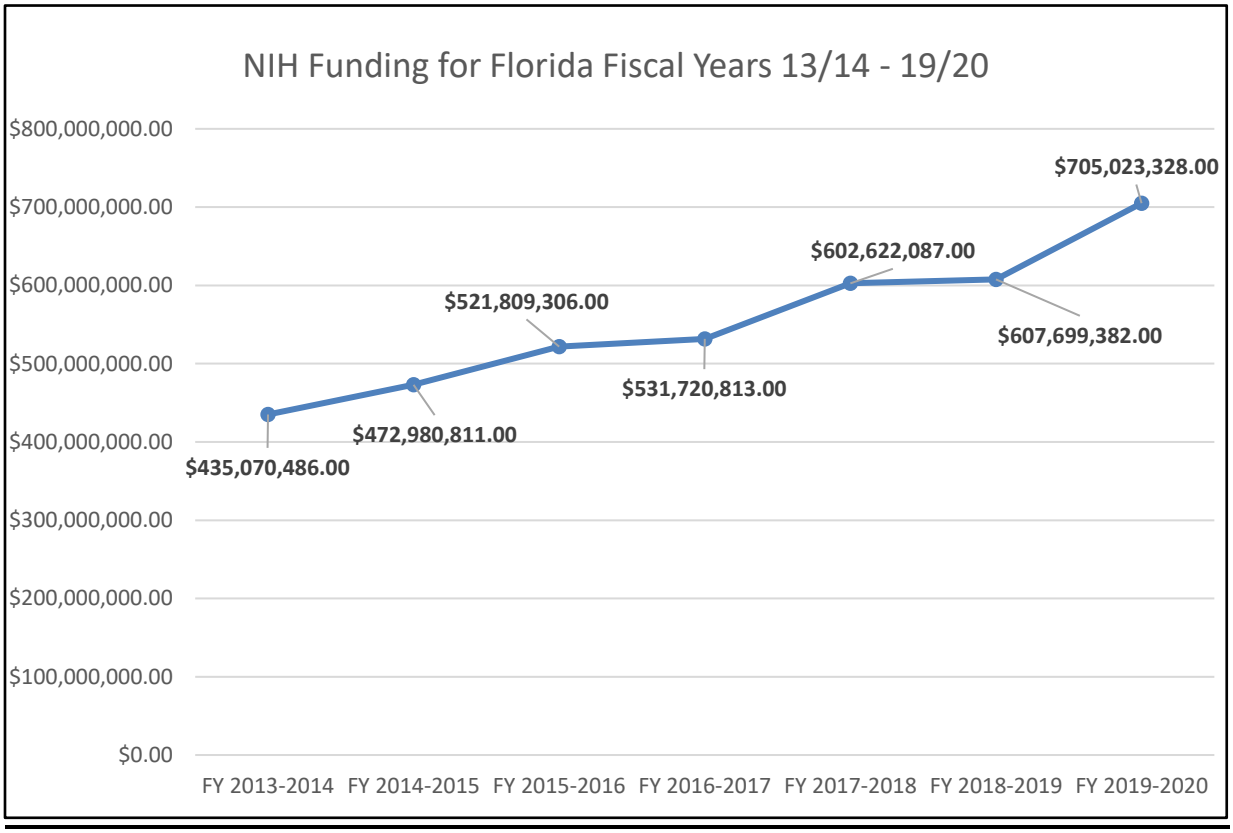
For the past four years, the state of Florida has remained 12th in the United States for federal funding. There was an increase in the total amount of funding for FY 2019-2020.

Figure 4: NIH Research Funding from the FY 2019 - 2020 Reporting Period

NIH Biomedical Research State Funding and Rankings FY 2019 - 2020		
State	**Total Funding**	**Rank**
CA	$4,591,581,664	1
MA	$3,024,098,902	2
NY	$2,891,776,354	3
PA	$1,944,017,304	4
MD	$1,920,138,523	5
NC	$1,589,742,045	6
TX	$1,370,180,699	7
WA	$1,135,332,133	8
IL	$1,012,456,813	9
OH	$883,089,814	10
MI	$826,532,093	11
FL	$705,023,328	12
MO	$655,608,101	13
GA	$630,030,140	14
MN	$619,407,697	15
CT	$603,000,869	16
TN	$585,280,713	17
VA	$506,061,472	18
WI	$493,137,164	19
CO	$42,723,332	20

Figure 4: The top 20 states in NIH funding is displayed. With over $705 million in NIH funding, Florida is ranked 12th in the nation. (*Source: NIH Research Portfolio Online Reporting Tools (RePORT).)*

Figure 5: NIH funding for Florida has increased to over $700 million. These results reflect Florida's initiative to expand upon research to improve scientific understanding of various diseases and health disparities.



Bankhead-Coley Cancer Biomedical Research Program
Appendix A
Fiscal Year 2020-2021 Newly Awarded Active Grants

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
20B01	All Children's Research Institute	Masanobu Komatsu, PhD	$636,611	$35,365	$601,246	5/19/20	4/30/23	No	No	No
20B02	Mayo Clinic Jacksonville	Derek Radisky, PhD	$99,999	$33,000	$66,999	5/1/20	10/31/20	No	No	No
20B03	Moffitt Cancer Center	Kenneth Shain, PhD, MD	$636,610	$0.00	$636,610	5/12/20	5/31/23	No	No	No
20B04	Moffitt Cancer Center	Paulo Rodriguez, PhD	$636,610	$0.00	$636,610	5/20/20	5/31/23	No	No	No
20B05	Moffitt Cancer Center		RELINQUISHED	$0.00	$0.00			No	No	No
20B06	Moffitt Cancer Center	Andry Marusyk, PhD	$636,610	$35,367	$636,610	5/5/20	4/30/23	No	No	No
20B07	Moffitt Cancer Center	Lixin Wan, PhD	$636,610	$0.00	$636,610	4/22/20	5/31/23	No	No	No
20B08	Moffitt Cancer Center	John M. Koomen, PhD	$253,555	$0.00	$253,555	5/15/20	5/31/23	No	No	No
20B09	Moffitt Cancer Center		RELINQUISHED	$0.00	$0.00			N/A	N/A	N/A
20B10	Moffitt Cancer Center	Nicholas J. Lawrence, PhD	$636,610	$0.00	$636,610	5/29/20	11/30/22	No	No	No
20B11	University of Florida	Elias J. Sayour, PhD, MD	$636,610	$0.00	$636,610	6/11/20	5/31/23	No	No	No
20B12	University of Miami	Sabitu Roy, PhD	$636,610	$0.00	$636,610	5/28/20	5/31/23	No	No	No
20B13	University of Miami	Jamie Merchan, MD	$636,610	$0.00	$636,610	6/16/20	5/31/23	No	No	No
20B14	University of Miami	Marzenna Blonska, PhD	$636,610	$0.00	$636,610	6/16/20	5/31/23	No	No	No
20B15	University of Miami	Lluis Morey, PhD	$636,610	$0.00	$636,610	6/5/20	5/31/23	No	No	No
20B16	University of Miami	Paulo Pinheiro, MSc, MD, PhD	$750,000	$0.00	$750,000	6/1/20	5/31/23	No	No	No
20B17	Moffitt Cancer Center	Jiandong Chen, PhD	$636,610	$0.00	$636,610	6/17/20	6/30/23	No	No	No

1. **Grant #:** 20B01 Reprogramming Tumor Immune Landscape by High Endothelial Venule Formation

   Principal Investigator: Masanobu Komatsu, PhD

   Organization: All Children's Research Institute, Inc./ Johns Hopkins University

   Abstract of Proposed Research: Checkpoint immunotherapies have recently become an extremely promising strategy for cancer treatment because this type of cancer therapy has shown the complete cure of malignant cancers in some patients. An important advantage of immunotherapy is that it causes relatively few significant side effects compared with chemotherapy. To date, however, only a fraction of patients has responded to immunotherapies in lung, breast, and other types of cancer. The success of checkpoint immunotherapy depends on patient's own immune cells, which fight against cancer cells and kill them. T-cells are immune cells primarily responsible for anti-tumor immune activity and have a potential to eradicate cancer cells. However, cancer cells create a type of environment around themselves that inhibits the T-cells from penetrating into the tumor interior. If our team can remodel the tumor environment in such a way that T-cells can penetrate the tumor, immunotherapies could work for most, if not all, patients. High Endothelial Venues (HEVs) are a type of blood vessels specialized for recruiting T-cells to the site of chronic inflammation. HEVs are also known to form in some cancer patients' tumors and serve as a gateway for T-cells to enter the tumor interior and destroy the cancer cells. Consistent with this T-cell recruiting role of HEVs, a high density of HEVs in patients' tumors strongly correlates with favorable clinical outcomes. These clinical observations suggest that enhanced HEV formation in tumors will create a richly T-cell infiltrated tumor environment and that such a reprograming of immune landscape renders tumors to be highly responsive to checkpoint immunotherapy. The mechanism of HEV formation is poorly understood at present. The new findings from our laboratory suggest that a Ras homolog protein R-Ras, which is expressed in the inner lining of tumor blood vessels, promotes the formation of HEVs in patients' tumors. Through comprehensive genetic analyses of HEVs, our staff also identified additional "signature" molecules of HEVs that are thought to be important for the regulation of HEVs. Our team hypothesize that the manipulation of these molecules would lead to abundant T-cell infiltration and greater anti-tumor T-cell immunity via increased HEV formation. In this proposal, our team will investigate these HEV signature molecules in human lung cancer and other types of cancer. Our team will establish correlations between their expression, abundance of HEVs, infiltrating immune cell profile, Th1/Th2 cytokine profile, and the retrospective clinical outcomes of each corresponding patient. In the short-term, the successful outcome of this study will determine how tumor vasculature may be targeted to generate highly inflammatory tumor environment that is sensitive to checkpoint immunotherapies. The current low response rate to checkpoint immunotherapy is one of the most important clinical challenges in cancer treatment. In the long-term, the new understanding gained from this study will allow the team to explore novel strategies to create immune stimulatory environment in patients' tumors, thereby making the non-responder patients to be responders of the immunotherapies. If this approach is found to be feasible and effective, there will be a direct and significant impact of such a finding to the care of cancer patients.

   Follow-on Funding: None at the time of reporting

   Collaborations: None at the time of reporting

   Journals: None at the time of reporting

Patents: None at the time of reporting

2. **Grant #:** 20B02 Involution-Based Biomarkers of Breast Cancer Risk

Principal Investigator: Derek Radisky, PhD

Organization: Mayo Clinic Jacksonville

Abstract of Proposed Research: Current risk prediction models for breast cancer rely on clinical and epidemiologic features. In other tissues, prediction of cancer susceptibility occurs optimally when a sample of the tissue at risk can be examined for evidence of premalignant change (e.g., cervix, colon). Over one million women per year in the United States undergo breast biopsies diagnosed as Benign Breast Disease (BBD), and yet beyond this catch-all diagnosis, biopsy findings are not effectively used to inform cancer risk models or clinical decisions. Importantly, these breast tissue biopsies contain critical information, at both morphologic and molecular levels, that could be used to predict later cancer susceptibility. One such morphologic tissue feature is Elucidation and Targeting of Novel Molecular Determinants of Tumor Progression and Dissemination the degree of age-related regression of background normal lobules seen in a breast biopsy. Our team has shown that Lithium (Li) is independently associated with reduced breast cancer risk, and the team hypothesize that delay of Li is linked to molecular mechanisms of carcinogenesis in postmenopausal women. In this proposed research, our staff will elucidate mechanisms controlling Li progression vs. delay and their association with progression to cancer in two BBD cohorts. In the Mayo Clinic BBD cohort, ~14,000 primarily Caucasian women had breast biopsies with benign findings, with ~1400 later breast cancers. In the Detroit BBD cohort of ~4,000 African American women, 222 breast cancers have occurred to date. For all these patients, our team have extensive information on the patients' clinical histories, the patient's benign biopsies, and the molecular features of the cancers which derived from them. If women at high risk for later breast cancer can be identified at the time of BBD, effective detection and prevention strategies can be pursued for these women. The work presented here is designed to identify why Li is delayed in many women, to identify the mediators of the processes that link delayed Li with breast cancer risk, and to reveal how this information could be used for improved risk assessment. In addition to immediate benefits from improved risk prediction, the proposed work has the potential to provide new insight into the driving forces underlying breast carcinogenesis. Most breast cancers occur in postmenopausal women, and incidence rates rise steeply during the perimenopausal years when the process is occurring. Investigations with the BBD cohort have revealed that breast cancer risk is concentrated in the 40% of postmenopausal women of whom the process of Li is delayed. Here, the team will specifically investigate peri- and post- menopausal women in whom the Li process is stalled or delayed defining the processes that govern Li and its connection to cancer risk. The team proposes that by focusing on these women, the team will discover novel insights about the relationship between aging and breast cancer. Moreover, it is expected that this information will provide insight into how Li may be activated in high risk postmenopausal women for physiological reduction of breast cancer incidence. At present, there is no information about why Li is delayed in so many postmenopausal women or why these women are at such increased risk for development of breast cancer, and these questions have now been a focus of study. The team believe that our expertise and novel tissue resource will allow our staff to fill this critical gap in knowledge, providing advance with direct clinical relevance.
Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

3. **Grant #:** 20B03 Development of Novel Cancer Drugs for the Treatment of Multiple Myeloma and Acute Myeloid Leukemia

   Principal Investigator: Kenneth Shain, PhD, MD

   Organization: H. Lee Moffitt Cancer Center and Research Institute

   Abstract of Proposed Research: Despite the introduction of new treatments, hematological (blood) cancers such as Multiple Myeloma (MM) and Acute Myeloid Leukemia (AML) remain largely incurable. Understanding how these cancers acquire resistance to treatment is essential if improved durable responses are to be realized. Pegylated Liposomal Doxorubicin (PLD) is a frontline therapy for MM. The anti-cancer action of PLD is mediated through modulating the activity of the DNA repair enzyme Topoisomerase II Alpha (TOP2A) resulting in catastrophic DNA damage and cancer cell death. Importantly, a common drug resistance mechanism in these cancers is the export of TOP2A from the nucleus into the cytoplasm, rendering PLD and other specific inhibitors of TOP2A ineffective.

   In previous studies the team demonstrated that the protein exportin1 (XPO1) binds TOP2A at a specific site called the nuclear export signal (NES) and actively exports TOP2A out of the nucleus into the cytoplasm in MM, AML and breast cancer cells. Notably, preventing XPO1 binding to the NES of TOP2A, retains TOP2A in the nucleus sensitizing cancer cells to PLD - induced death. Our team have demonstrated that inhibitors of XPO1 prevents TOP2A export from the nucleus and sensitizes MM cells to PLD and other chemotherapies. However, XPO1 mediates the nuclear export of hundreds of cellular proteins and inhibition of XPO1 may result in unwanted toxic side effects. Our team therefore searched for a NES inhibitor (NESi) that would specifically bind to the NES of TOP2A preventing the nuclear export of only TOP2A. The protein sequence of the NES of TOP2A is unique and could lead to the development of drugs that exclusively block the NES of TOP2A and not affect XPO1-dependent export of other nuclear proteins. Using a computer model of the 3-dimensional structure of TOP2A protein the team screened 139,735 small molecules from the NCI database to assess whether any would block the NES of TOP2A. From this compound library our staff identified three that were effective in sensitizing MM to PLD. For example, NCI-9138 prevented TOP2A-XPO1 binding and the selective export of TOP2A when tested in MM cells. When combined with the TOP2A inhibitor PLD, NCI-9138 synergistically killed MM cells in culture and MM cells isolated from patient bone marrow samples with minimal effects observed on normal non-cancerous cells. The focus of this proposal is to improve the anti-cancer activity of the three compounds identified in our search of the NCI database by specific modifications their chemical structures. Using structure- guided design, an iterative medicinal chemistry approach and a battery of biochemical, biophysical (SPR, NMR and co- crystallography) and cell-based assays integrated within a rigorous research operating plan (ROP), the team will enhance the potency of the NES inhibitors. NESi analogs with improved drug-like properties will be tested in bone marrow cells isolated from MM patients and in two mouse models, one with human MM tumors and a mouse MM bone marrow model. Patient MM cells will be examined for markers that predict response to the therapy, which is essential for NESi in future clinical trials.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

4. **Grant #:** 20B04 Notch Signaling Boosts T-Cell Based Immunotherapy

   Principal Investigator: Paulo Rodriguez, PhD

   Organization: H. Lee Moffitt Cancer Center and Research Institute

   Abstract of Proposed Research: Malignant epithelial tumors, including melanoma and lung carcinoma, create a chronic inflammatory milieu that impairs anti-tumor immunity, thereby decreasing the potential benefit of different types of cancer treatment. Indeed, the highly immunoinhibitory and metabolically constraining Tumor Microenvironment (TME) represents a major limitation for the curative effect of T-cell -based immunotherapies, including Tumor-Infiltrating T-cells (TILs) and Chimeric Antigen Receptor (CAR) T-cells. The expansion of heterogeneous subsets of immunosuppressive myeloid cells in the TME de-activates endogenous and adoptively transferred T-cells, thereby driving their functional suppression. Therefore, development of strategies to render adoptively transferred T-cells refractory to the TME, especially to tumor- infiltrating myeloid cells, could enable the implementation of the next generation of T-cell based therapies that effectively eradicate solid tumors. Our preliminary reports showed the primary role of the transcription factors, Notch1-2, in the function of T-cells. Overexpression of Notch1 intracellular active domain (N1IC) in T-cells, or expansion of T-cells in the presence of high-affinity variants of Notch ligand-DLL4, boosted their anti-tumor effects after adoptive transfer immunotherapy, which correlated with an increased mitochondrial fitness, higher levels of major anti-oxidant mediators, and expansion of Tissue Resident Memory (TRM) T-cell, a subset of tumor- reactive T-cells that resist metabolic restrictions and inhibitory signals at the TME. Interestingly, Notch stimulation in CD8+ T-cells conferred resistance to myeloid-derived suppressive cells (MDSC) related immunosuppression. Our team hypothesize that activation of the Notch pathway makes endogenous and adoptively transferred T-cells highly efficient at eliminating tumors through promotion of effector signals, differentiation into TRM T-cells, and induction of primary drivers that confer resistance to immunoinhibitory myelopoiesis in the TME. Thus, using adoptive transfer of tumor-specific T-cells and a recently developed model of Chimeric Endocrine Receptors (CER), our team will combine expertise in Notch activity and T-cell based therapies to determine the therapeutic effect of the modulation of Notch signaling in adoptively-transferred T-cells in mouse models of melanoma, lung carcinoma, and ovarian carcinoma. Our team propose the following Specific Aims: Aim 1: Define how the modulation of Notch signaling significantly improves the therapeutic potential of adoptively transferred T-cells in models of melanoma, lung and ovarian cancer. Aim 2: Elucidate the mechanisms whereby Notch-active T-cells become refractory to myeloid cell -driven immunosuppression in tumors. Aim 3: Define concurrent genetic interventions that synergize with ectopic Notch activity in transferred T-cells. Information gathered from this effort is expected to provide insights into the central mechanisms underlying T-cell de-activation in tumors, which could lead to the development of the next generation of T-cell based therapies highly effective in solid malignancies.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

5. **Grant #:** 20B06 Impact of Stromal Architecture on the Response of Lung Cancers to Targeted Therapies

Principal Investigator: Andriy Marusyk, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract of Proposed Research: While highly effective, targeted therapies are not curative in advanced metastatic lung cancers, as most tumors eventually develop resistance and relapse. In most documented cases, the resistance is linked to specific mutations which confer cell-intrinsic resistance by either reducing the ability of drugs to shut down their targets or make tumor cells less dependent on the original oncogene. From the analyses of durations of responses to therapies, and from the growing body of experimental studies, it appears that, in most cases, resistance is acquired during treatment by tumor cells that, initially, can barely survive the treatment. A higher numbers of tumor cells surviving treatment translates into higher odds that some of them will eventually acquire resistance associated mutations, which will drive relapse. While some tumor cells may survive due to intrinsic characteristics, others might be able to survive only because the cells receive external pro-survival signals and cytokines secreted by stromal (non-cancerous) cells. Cancer Associated Fibroblasts (CAFs), the most abundant cell type in tumor stroma, appear to be especially important in this regard. Experimental studies involving either co-culture of tumor cells with fibroblasts, isolated from tumors, or supplementation of CAF-produced factors into tumor cell culture media, demonstrated that CAFs could dramatically blunt the effect of targeted drugs, including inhibitors of Epidermal Growth Factor Receptor (EGFR) and Anaplastic Lymphoma Kinase and (ALK) in lung cancers. However, despite the accumulation of in vitro evidence for CAF mediated stromal protection, its relevance to what is happening in real tumors remains unclear. CAFs that grow in culture dishes do not fully recapitulate their counterparts within tumors. More importantly, whereas in vitro tumor cells are usually well mixed with fibroblasts (or exposed to same concentrations of CAF-produced survival factors), their counterparts in vivo experience a much more uneven distribution of protective environments. Stroma and cancer cells form spatially distinct clusters. As a result, tumor cells that reside close to stroma could experience an abundance of pro-survival signals, while those tumor cells that reside far from stroma could get little or none. Different tumors have vastly different patterns of topology of stroma and tumor cells. Therefore, the degree of inequality in terms of the access to the putative pro-survival signaling, is also highly variable. Our proposal will address two key questions. First, the team will define how much stromal cells protect tumor cells from the effects of the drugs *in vivo*, elucidating the relationship between the spatial location of tumor cells relative to stroma, and their probability to die and to divide in the presence and absence of treatment. To address this question, our team will develop new digital image analysis tools, re-tooling approaches from the area of spatial ecology, and from the modeling of spatial physical diffusion processes. Second, our team will address how the different patterns of spatial distribution of tumor cells and stroma affect the depth of the initial response of lung cancers to targeted therapies. Successful

completion of our studies will improve our ability to predict response of tumors to therapies, and opens doors to the development of therapy approaches, based on the understanding of the evolutionary dynamics.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

6. **Grant #:** 20B07 Characterizing Oncogenic Function of the Itchy E3 Ubiquitin Protein Ligase (ITCH) in Melanoma

   Principal Investigator: Lixin Wan, PhD

   Organization: H. Lee Moffitt Cancer Center and Research Institute

   Abstract of Proposed Research: The combination of BRAF gene and Methyl Ethyl Ketone (MEK)-targeted therapies with anti- Programmed Death Cell Protein 1 (PD-1) immunotherapy is now the standard of care for patients of BRAF-mutated melanoma. For BRAF wild-type melanoma and other types of solid tumors, however, MEK inhibitor monotherapy often shows little advantage over chemotherapies. Thus, new strategies for targeted therapy combination are urgently needed. The Itchy E3 Ubiquitin Protein Ligase (ITCH) gene has been well characterized as a key molecule in immune cells. Itch knockout mice develop autoimmune phenotypes through several mechanisms modulating both T-cell and B-cell functions, and the gene was therefore named after the itchy phenotype. On the other hand, roles of ITCH in tumorigenesis, especially in the in vivo settings, are less explored. The team has recently uncovered that ITCH promotes BRAF activation in response to proinflammatory cytokines in BRAF wild-type melanoma cells. ITCH deficiency led to a drastic reduction of viability in BRAF wild-type melanoma cells both in culture and in the mice. Interestingly, BRAF wild-type mouse melanoma cells developed smaller tumors in Itch knockout mice. Based on our recently published as well as preliminary data, the team hypothesize that that in BRAF wild-type melanoma cells, ITCH is a therapeutic vulnerability in both melanoma cells and the tumor immune microenvironment. In melanoma cells, pro-inflammatory cytokines activate the Jun N-Terminal Kinase (JNK)-ITCH pathway to sustain oncogenic pathways including MAPK and MITF; Activation of ITCH in immune cells, on the other hand, fosters an immunosuppressive tumor microenvironment. Hence targeting ITCH could be novel strategy to kill two birds with one stone Inhibiting ITCH function may also sensitizes MEK and immune checkpoint inhibitors in BRAF wild-type melanomas. To test our hypothesis, the team aims to: 1) define mechanisms by which ITCH facilitates melanoma cell proliferation and migration; 2) determine if activation of ITCH in melanoma cells promotes tumorigenesis; 3) assess the oncogenic roles of ITCH in melanoma cell survival and immune evasion in vivo. The completion of these studies will provide a strong basis for our long-term goals to develop small molecules specifically inhibiting ITCH, to assess targeting ITCH is also an effective strategy in other RAF-activated solid tumors, and to translate our discoveries into new investigator initiated clinical trial.

   Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

7. **Grant #:** 20B08 Proteogenomics of Metastatic Heterogeneity and Therapeutic Resistance in Lung Cancer

   Principal Investigator: John Koomen, PhD

   Organization: H. Lee Moffitt Cancer Center and Research Institute

   Abstract of Proposed Research: Lung cancer has limited treatment options for advanced, recurrent, or metastatic disease. Frontline treatment has traditionally consisted of chemotherapy, but targeted drugs (e.g. EGFR and ALK inhibitors) and immunotherapy are showing significant impact in improved patient outcomes. However, no strategy is currently curative, so our team must study advanced lung cancers resistant to these therapies to develop molecular classifiers of response and resistance as well as design novel treatment strategies. The first step is to elucidate the biology of drug resistant lung tumors. To support this goal, our research team consents patients for rapid tissue donation after autopsy. Multiple metastatic lesions from nine patients that died from cancer (three small cell lung cancer, six adenocarcinoma) have been collected and annotated with clinical data, including serial radiology studies documenting the response or resistance to therapy of each tumor site. The diagnoses and treatment regimens for example patients are a small cell lung cancer patient treated with chemotherapy, a KRAS mutant adenocarcinoma patient treated with immunotherapy and targeted therapy, and an Anaplastic Lymphoma Kinase (ALK)-positive adenocarcinoma patient treated with chemotherapy, immunotherapy, and ALK inhibitors. Our research team will explore changes associated with metastasis and address the hypothesis that tumor drivers and support mechanisms differ between lung tumors and distant metastases. Proteogenomic and support mechanisms differ between lung tumors and distant metastases. Proteogenomic includes sequencing to detect mutations and fusions, gene expression measurements from RNASeq, expression proteomics, and quantification of protein biomarker panels. Mutation patterns from genomics will define the sequence of spread throughout the body to establish the natural history of the disease. To define specific adaptations to metastatic niches and microenvironmental support mechanisms in each organ site, the team will compare proteogenomic changes between primary lung tumors, local lung metastases, and metastases to other organs. Furthermore, our team will test the hypothesis that response or resistance to each type of treatment is determined by the adaptations and microenvironment of each metastatic tumor. The team will focus initially on 1) Conventional chemotherapy-DNA damage response elements and detoxifying enzymes 2) Immunotherapy-tumor mutation burden, infiltrating immune cells, immune checkpoint proteins and ligands 3) Targeted Therapy–kinases.

   Also, bioinformatics approaches will identify changes in genes and proteins relevant to resistance to each type of treatment to improve our understanding of evasion of therapy and the reasons why these tumors were fatal to the patient. The primary endpoint of this study is to increase our knowledge of metastatic and drug resistant lung tumors and confirm these candidate biomarkers with pathology follow up experiments; secondary goals include gene/protein correlations, biomarker development with proteomics or immunohistochemistry, and targetable vulnerabilities. In summary, our team expects to improve our knowledge of lung cancer, particularly

emphasizing signaling, heterogeneity of different metastatic lesions growing in the same patient, and phenotypes of drug resistant tumors to define novel vulnerabilities that can be assessed in the patient and targeted with novel treatment strategies for metastatic tumors with companion biomarkers that predict their success.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

8. **Grant #:** 20B10 Novel Monovalent and Bivalent JAK2 Inhibitors for Targeted MPN and Cancer Therapies

Principal Investigator: Nicholas Lawrence, PhD

Organization: Moffitt Cancer Center / University of South Florida

Abstract of Proposed Research: Myeloproliferative neoplasms (MPNs) are chronic leukemias composed of three main phenotypes including polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (MF). MPN prevalence estimates that over 300,000 United States patients live with the disease and all of patients with PV having one of several JAK2 mutations and 60% of MF and ET patients having a JAK2 mutation. For this reason, focusing on JAK2 as a target for treating myeloid cancers has gained considerable interest. Clinical JAK2 inhibitors such as ruxolitinib for MPNs have high potency and improve patient survivability, but show quick adaptive resistance leading to the prevention of remission of MPNs. Current JAK2 inhibitors often cause severe adverse reactions due to off-target effects limiting treatments to approximately one year with no signs of remission. To overcome these challenges novel drug therapies are needed to better combat MPNs and prevent drug resistance. This project proposes the development of multi-targeted agents that may have numerous advantages over current inhibitors by exerting increased efficacy in MPN models and high selectivity for cells driven by oncogenic mutant JAK2. Specific Aim 1 describes the optimization of single drugs able to potently and simultaneously inhibit JAK2 and bromo domain- containing protein 4 (BRD4). Recent findings by our labs have led to orally available dual BRD4-JAK2 inhibitors with high efficacy in MPN models. Additionally, resistance to dual-target inhibitors through concurrent mutations in both target proteins did not develop. Ten lead compounds will be further investigated for drug metabolism and pharmacokinetic properties, and efficacy in MPN animal models and patient samples. These drugs will also serve for the generation of Proteolysis-Targeting Chimeras (PROTACs) to aid in the degradation of mutant JAK2 and BRD4. Specific Aim 2 describes the development of novel bivalent inhibitors and PROTACs based on our recently determined co-crystal structure of JAK2 with ruxolitinib. Compounds have been designed to promote JAK2 dimerization for improving mutant selectivity as well as inducing the autoinhibited conformation of JAK2 to remove the constitutive activity of the mutant enzyme. Additionally, PROTACS will be generated to target the mutant JAK2 oncoprotein for degradation. Our team proposes an interdisciplinary approach toward the development and characterization of target specific markers of MPNs using medicinal chemistry, biochemistry and cell and animal models to improve efficacy and effectiveness over current clinical treatments. The proposed research is original, innovative, and set to establish a means of dramatically increasing selectivity for disease causing forms of

JAK2. Progress in our studies may also benefit other JAK based diseases, such as rheumatoid arthritis. The kinase inhibitory profile of the compounds from Specific Aim 1 also suggests potential activity in solid cancers such as prostate and lung.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

9. Grant #: 20B11 Lipid-Nanoparticle Vaccines Targeting Metastatic Lung Cancer from Osteosarcoma

Principal Investigator: Elias Sayour, PhD, MD

Organization: University of Florida

Abstract of Proposed Research: Despite multi-modality approaches for osteosarcoma (OS), including chemotherapy and limb amputation, a significant percentage of children/adolescents succumb to disease due to the presence of lung metastasis; these outcomes necessitate development of novel targeted therapeutics. Immunotherapy promises to re-direct the host immune system against OS but remains limited by the dearth of antigen specific targets and the immunosuppressive tumor microenvironment. To circumvent the lack of OS specific targets and overcome intra-tumoral immunosuppression, our group has developed a novel treatment platform that consists of clinically translatable nanoparticles (NPs) combined with personalized tumor derived mRNA. These RNA-NPs can simultaneously function as both a vaccine and an innate immunomodulating agent to reprogram OS mediated immunosuppression into an immune activated milieu. The team has shown that intravenous administration of tumor mRNA loaded NPs transfect antigen presenting cells and lead to an activated T-cell response for induction of anti-tumor immunity in preclinical models. In contrast to other vaccine formulations, RNA-NPs recruit multiple arm of the immune system (i.e. innate and adaptive), and remodel the systemic/intra-tumoral immune milieu, which remain potent barriers for vaccine, cellular, and checkpoint inhibiting immunotherapies. In murine pulmonary OS models, RNA-NPs induce robust anti-tumor efficacy (~87.5% long-term survivor benefit) and mediate synergistic activity in settings where immune checkpoint inhibitors (i.e. anti-PD-L1 therapy) do not confer therapeutic benefit. Drs. Sayour and Heldermon will explore mechanisms of treatment resistance in syngeneic immunocompetent murine models for metastatic OS (MOS-J and K7M2). Drs. Sayour and Heldermon will then use the nanoparticle delivery strategy to target identified mechanisms before pursuing a translational canine study exploring the safety and activity of combination RNA-NPs in canines with OS (nearly 100% homologous to the human form of the disease).

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of funding

Journals: None at the time of funding

Patents: None at the time of funding

10. **Grant #:** 20B12 Targeting the Gut Microbiome to Improve Cancer Pain Management by Opioids

    Principal Investigator: Sabita Roy, PhD

    Organization: University of Miami

    Abstract of Proposed Research: This is a resubmission of a previous proposal investigating the role of the gut microbiome in cancer associated pain in metastatic breast cancer. Bone metastasis is associated with severe pain and dramatically diminishes quality of life. During cancer progression, inflammatory mediators are elevated in patients with metastatic breast cancer. Very few studies have investigated the role of the gut microbiome in breast metastatic cancer and how it contributes to cancer-associated pain. Preliminary studies have established that gut permeability changes and dysbiosis of gut microbiome result in sustained inflammation. Inflammation causes chronic pain. The team's overarching hypothesis is that alterations in the gut microbiome associated with metastatic cancer leads to induction of inflammatory cytokines thereby, contributing to chronic pain. Opioid treatment is the gold standard for pain management in cancer patients. Unfortunately, the efficacy of opioids in long term pain management is limited because of the development of tolerance with chronic use. Our team's recent studies have established that alterations in the gut microbiome with sustained inflammation limits the analgesic effects of morphine and leads to the development of tolerance to opioids. The team hypothesize that long term administration of prescription opioids for pain management in patients with metastatic breast cancer will have limited efficacy because of accelerated tolerance development. In preliminary data, the team showed that probiotics treatment prolonged opioid efficacy in pain management and delayed development of tolerance. Based on these studies, our team propose that treatment with probiotics in patients with cancer pain will reduce inflammation associated with pain and prolong the efficacy of opioids for pain management. In Aim 1, the team will establish that alteration in the gut microbiome contributes to pain due to sustained pro-inflammatory immune cell activation in a murine model of metastatic breast cancer. In Aim 2, the team will establish that opioids have limited efficacy in controlling pain in metastatic breast cancer because of rapid tolerance development as a consequence of microbial dysbiosis. In Aim 3, team will establish that designer probiotics that replaces selectively depleted bacterial communities in metastatic breast cancer will attenuate pain associated with metastatic cancer and prolong the efficacy of opioids as an analgesic agent.

    Follow-on Funding: None at the time of reporting

    Collaborations: None at the time of reporting

    Journals: None at the time of reporting

    Patents: None at the time of reporting

11. **Grant #:** 20B13 Tumor and Stromal Targeted Oncolytic Virus based Biotherapies for Colorectal Cancer

    Principal Investigator: Jaime Merchant, MSc, MD

Organization: University of Miami

Abstract of Proposed Research: Despite major advances in the therapeutic landscape in advanced colorectal cancer (CRC), the majority of patients still succumb from progressive disease. Advanced CRC rapidly develops resistance to standard chemo and targeted therapies, and the great majority of cases do not respond to checkpoint inhibitors alone. Therefore, development of novel biotherapies able to overcome resistance to currently available CRC agent is an urgent medical need. Oncolytic viruses (OVs) are promising biological antitumor agents that offer an advantage over conventional oncology drugs, because the cells can be genetically engineered to target, replicate in, and kill tumor cells. In addition, the cells may also act as immunomodulatory agents. Among the different OV platforms currently being developed, the oncolytic Measles Virus (MV) is a promising one, as it has shown safety and antitumor activity in vitro, in vivo and in early phase clinical trials. There are several obstacles, however, limiting the efficacy of systemically administered OVs. The cells include inadequate viral entry due to the tumor stromal barrier, limited viral cytotoxicity due to decreased tumor viral replication, and short lived viral induced antitumor immunity. Our long-term goals are to overcome this barrier by developing stromal re-targeted oncolytic measles viruses and to improve viral oncolysis by combination novel antitumor agents. The team generated preliminary data showing that novel oncolytic MVs fully retargeted against human or murine urokinase receptors has significant antitumor effects in vitro and in vivo models of colorectal cancer by targeting both the tumor and importantly, the stromal cells compartment of the tumor. While significant antitumor effects were observed, complete responses were not observed. In preliminary studies, the team found that combination of oncolytic MVs and the novel agent triptolide lead to significant increase in tumor cytotoxicity in vitro, probably by enhancing viral replication and inducing increased tumor cell apoptosis. This is a novel, promising combination that may significantly improve antitumor responses in colon and other cancers. The objectives of the application are to further characterize the mechanisms by which OV stromal targeting enhances the virus' overall oncolytic effects, and to further evaluate the effects and molecular mechanisms of the virus-triptolide combination in models of colorectal cancer. The team propose to achieve our objectives by pursuing three aims. 1) To characterize the mechanisms of tumor -stromal interactions in colorectal cancer models; this will be pursued by characterization and molecular analysis of stromal cell components before and after treatment in vitro and in vivo. 2) To test the in vivo efficacy of tumor, stromal or dual targeted oncolytic MVs in combination with minnelide in CRC xenografts and syngeneic models of CRC. 3) To characterize the in vivo effects of MV-muPA on the immune stroma and their modulation by minnelide, checkpoint inhibitors or both in syngeneic CRC models. The above studies will bring significant new knowledge in the field of stromal targeted oncolytic viruses and has the potential to bring a novel new combination strategy for the treatment of this fatal disease. Our extensive preliminary data, as well as the resources, experience and expertise the team has gained in the last eight years in the OV field provide assurance that team will successfully achieve the above aims and objectives of the application.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

12. Grant #: 20B14 Elucidation and Targeting of Novel Molecular Determinants of Tumor Progression and Dissemination
Principal Investigator: Marzenna Blonska, PhD

Organization: University of Miami

Abstract of Proposed Research: The molecular and genetic features that drive the aggressive clinical behavior of B-cell lymphoma have not been fully defined. In addition, there are very limited data regarding the cellular and molecular interactions that facilitate lymphoma dissemination, including trafficking of lymphoma cells to the brain. Our gene expression profiling of lymphoma cells revealed the elevated levels of forkhead box C1 (FoxC1) transcription factor in the activated B-cell like subtype of Diffuse Large B-Cell Lymphoma ABC-DLBCL), the disease with the worst prognosis. Importantly, high levels of FoxC1 correlated with disseminated disease and multi-organ involvement. Our findings suggest that FoxC1 play the important role in lymphoma dissemination and/or growth in secondary (extra-nodal) sites. In addition, overexpression of FoxC1 seems to be a constant feature of the central nervous system lymphoma. However, it remains to be investigated whether FoxC1 promotes brain colonization or/and supports lymphoma cell growth in the brain environment. To date, the specific physiological function of FoxC1 has not yet been determined, however it has been shown to play a role in the regulation of embryonic and ocular development, and it is essential for the brain development. To date aberrant expression of FoxC1 has been detected in 17 solid tumor types including: basal-like breast cancer, melanoma, and non-small cell lung cancer, and it is associated with poor prognosis. Our current study demonstrates, for the first time, that FoxC1 can be aberrantly expressed in lymphoma cells. The biological role of FoxC1 in malignant lymphocytes has not been studied and the genes regulated by FoxC1 at the transcriptional level remain to be determined. It is also not clear whether FoxC1 can function as a driver of metastatic disease or biomarker for solid tumors and lymphoma. To address these emerging questions, our team generated two novel mouse models: (a) Xenograft model of ABC-DLBCL with spontaneous dissemination of lymphoma cells into extra-nodal sites including the brain; (b) FoxC1 conditional transgenic mice with tissue-specific and inducible expression of FoxC1. Our mice will be crossed with several different mouse models of lymphoma and solid tumors. In this study, our team propose three specific aims: 1) To determine the role of FoxC1 in dissemination of lymphoma and breast cancer in vivo; 2) To identify the genes that are controlled by FoxC1 in both lymphoma and solid tumor cells; 3) To identify (high- content screening of a large library of kinase inhibitors) and validate the compounds that suppress expression, activation, and nuclear translocation of FoxC1 in malignant cells. Top inhibitors will be tested both in vitro and in vivo. Together, in this project, the team will define the role of FoxC1 and FoxC1-regulated genes in the aggressive tumors and the mechanism of brain colonization. Our study will provide a rationale for screening lymphoma patients for the expression level of FoxC1 using either genetic or histological methods. The preclinical testing of FoxC1 inhibitors will lead to designing a novel therapeutic intervention in patients with aberrant expression of FoxC1 with various tumors.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

13. Grant #: 20B15 Mechanisms of Polycomb Complexes in Luminal Breast Cancer

Principal Investigator: Luis Morey, PhD

Organization: University of Miami

Abstract of Proposed Research: About one in eight U.S. women will develop invasive breast cancer over the course of the women's lifetime. Breast cancer can be classified by the presence or absence of hormone receptors including the estrogen receptor alpha (ERα). Over two thirds of breast cancers express ERα, and despite tremendous advances in breast cancer treatment, 50% of patients do not respond to therapies. Currently, modulators and degraders of ERα, such Tamoxifen and Fulvestrant, are treatment options for patients with ER+ breast cancer. However, a large percentage of cases exhibit resistance or acquire resistance to estrogen inhibitors, resulting in colonization of distal organs and metastasis. Interestingly, patients with metastatic ER+ breast cancer often harbor a point mutation in ESR1, the gene encoding for ERα. Importantly, ESR1 mutations are only found in metastatic sites and not in primary tumors, suggesting that clonal expansion of ESR1-mutant cells might promote metastasis. These observations indicate that ERα is required for both initiation and progression in ER+ breast cancer. Therefore, development of new agents aimed at reversing resistance to hormonal therapies and to improve current treatment are very much desired. There is increasing evidence that epigenetic machineries are deregulated in cancer. The team has recently showed that the Polycomb gene RNF2 (encoding RING1B) is significantly overexpressed in newly diagnosed breast cancer. RING1B is a core subunit of the Polycomb Repressive Complex 1, which is classically associated to gene repression. There are dozens of PRC1 variants, named canonical and non-canonical (cPRC1 and ncPRC1), which are defined by their molecular composition. PRC1 complexes compact chromatin and mono-ubiquitinate lysine 119 on histone H2A (H2AK119ub1) by the E3-ligases RING1A or RING1B. Our team and others showed that the architecture of PRC1 complex is dynamic during early development and that PRC1 variants have specific biological functions. Our results provided the first indication that PRC1 specific gene targets, recruitment mechanisms, and enzymatic activities are context dependent, and that their expert specialized and distinct functions during the early stages of development. How PRC1 complexes control genomic programs in cancer has only recently attracted attention. In contrast to its canonical role as a gene repressor, the team recently showed that the core PRC1 subunit RING1B positively controls the transcriptional activity of genes regulated by estrogen and ERα iER+ breast cancer, revealing an unforeseen epigenetic-mediated mechanism controlling the estrogen pathway. Moreover, the team has now found that RING1B is overexpressed in patients with treatment-resistant metastatic ER+ breast cancer, and that RING1B depletion sensitizes cells to endocrine treatment. Our published and new results indicate a functional relationship between Polycomb complexes and ERα and represents a promising avenue to discover new therapeutic options for treating ERα- dependent breast cancers. The team propose that modulation of the Polycomb functions in ER+ breast cancers may yield a candidate new therapeutic option for breast cancer patients.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

14. Grant #: 20B16 Risk, Etiology, and Mortality for Highly Fatal Cancers in Diverse Florida; Unique Impact on African Americans, Afro-Caribbean's, Cubans, Puerto Ricans and other Hispanics

Principal Investigator: Paulo Pinheiro, PhD

Organization: University of Miami

Abstract of Proposed Research: Lung cancer is the leading cause of cancer death and liver cancer is the fastest growing cancer, both in the United States (US) and the Sunshine state of Florida, (population 21 million) where combined these cancers account for over 13,000 deaths each year. Unfortunately, survival prospects are dismal for both diseases. Currently, only 18% of liver cancer patients and 17% of lung cancer patients survive five years. Lung cancer mortality is highest among African Americans while liver cancer disproportionately affects all non-White minorities. However, the rich racial/ethnic diversity in Florida has yet to be leveraged to explore the underlying etiologies and clinical and biological characteristics impacting mortality. To address these disparities, knowledge about the specific risk factors land etiologies driving these cancers for each distinct population in Florida needs to be clarified so that actionable public health and clinical interventions can take place. The team proposes to study the lung and liver cancer experience for 12 distinct racial/ethnic groups in Florida, beginning with the typically studied major groups, White, Black and Hispanic, but also distinct subgroups with sizable populations in Florida, including African American (US-Born), Afro-Caribbean, Cuban, Puerto Rican, Mexican, Dominican, Central American, and South American. This knowledge will enable the development of specific approaches for high risk groups, including tobacco control, and access to curative treatment for lung cancer, and screening underlying liver disease and for hepatitis B and/or C for liver cancer. Our first aim is to determine the incidence and survival of lung cancer for each race/ethnicity and to study and identify the clinical, biological, and social determinants of lung cancer survival for all 12 populations. Sub-analyses will include an in-depth assessment of lung cancers among non-smokers, rarely studied but with a heavier impact among racial/ethnic minorities and women, as well as the identification of any disparities in the receipt of surgical treatment for localized NSCLC (non-small cell lung carcinoma) tumors, most amenable to cure. Our second aim is to determine cause specific liver cancer incidence and survival, for both major types of liver cancers, HCC (hepatocellular carcinoma) and ICC (intra-hepatic cholangiocarcinoma), examining the known etiologies of liver cancer: hepatis C, hepatis B, alcohol liver disease, NAFLD, diabetes, and rarer genetic, autoimmune/inflammatory and biliary conditions. Special emphasis will be placed on the patterns among baby boomers, known to have higher HCV prevalence and a higher liver cancer burden. Florida's hospital discharge data, viral hepatitis and mortality data will be linked with cancer registry data to provide unique etiology- specific information for a large, representative statewide cohort of Hispanic and Black minority subgroups, never studied with this level of detail anywhere else in the US. Over 200,000 lung and 22,000 liver cancer cases over the course of 13 years (2005-2017) have been preliminarily identified using the Florida Cancer Data System. This proposal will use unique data and innovative multisource linkages to produce entirely novel information about Florida's diverse cancer profile. Community outreach to disseminate our results will provide invaluable information for both public health and clinical practitioners, tasked with combatting the scourge of these two deadly cancers.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting
Patents: None at the time of reporting

15. Grant #: 20B17 Discovery of p53 Inhibitors for Reducing Toxicity of Chemotherapy

Principal Investigator: Jiandong Chen, PhD

Organization: Moffitt Cancer Center

Abstract of Proposed Research: DNA-damaging chemotherapy drugs induce arrest or apoptosis of tumor cells partly through activating p53. However, these drugs also activate p53 in normal tissues, causing organ damage and dose-limiting toxicity. Chemotherapy of tumors with p53 mutation (>50% of cases) no longer benefit from p53 activation in tumor cells, but the toxicity to normal tissues remains due to presence of wild type p53. P53-mediated apoptosis is also implicated in stroke-related tissue damage and neuro-degenerative diseases. Therefore, in certain clinical settings, short-term inhibition of p53 activity may reduce organ damage and increase tolerance to high-dose chemotherapy. Currently, there are no drugs or chemical probes that can specifically inhibit the activity of p53, thus hampering the investigation of this hypothesis. To address this issue, our team developed a high throughput screen (HTS) assay for compounds that inhibit p53 binding to DNA. The team identified two inhibitors of p53 DNA binding in a pilot screen. To further advance this research, the team propose the follow aims: (1) Perform a 170,000 compound HTS for inhibitors that inhibit DNA binding by activated p53; (2) Improve the activity of existing and new hits through chemical modification and functional analysis. This work will identify lead compounds to inhibit p53 and protect sensitive tissues during chemotherapy and validate an approach to target “non-druggable” transcription factors.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

Bankhead-Coley Cancer Biomedical Research Program
Appendix B
Fiscal Year 2019-2020 Active Grants
Funding Fiscal Year 2018-2019

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditures	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow on Funding
9BC01	Florida Atlantic University	Guzman, Esther, PhD	$801,000	$222,500.00	$578,500.00	5/10/2019	5/31/2022	No	No	No
9BC03	Florida State University	Steiner, Jennifer MS, PhD	$732,238	$264,420.00	$467,818.00	6/17/2019	5/31/2022	No	No	No
9BC04	Florida State University	George Rust, MD	$800,487	$222,350.00	$578,137.00	5/28/2019	5/31/2022	No	No	No
9BC07	H. Lee Moffitt Cancer Center	Gina M. DeNicola, PhD	$1,335,000	$222,500.00	$1,112,500.00	4/17/2019	5/31/2024	No	Yes	No
9BC08	H. Lee Moffitt Cancer Center	Nelli Bejanyan, MD	$1,335,000	$267,000.00	$1,068,000.00	4/17/2019	3/31/2024	No	No	Yes
9BC09	H. Lee Moffitt Cancer Center	Ernst Schonbrunn, PhD	$800,454	$333,525.00	$466,929.00	3/26/2019	3/31/2022	No	No	No
9BC12	University of Miami	Anthony Capobianco, PhD	$801,000	$178,000.00	$623,000.00	5/7/2019	4/30/2022	No	No	No
9BC13	University of Miami	Kerry Burnstein, PhD	$801,000	$178,000.00	$623,000.00	4/23/2019	4/30/2022	No	Yes	No
9BC14	University of South Florida	Hong Yuan (Rays) Jiang, PhD	$801,000	$311,500.00	$489,500.00	6/4/2019	4/30/2022	No	No	No

1. Grant #: 9BC01 Discovery of Marine Natural Products Active Against Triple Negative Breast Cancer Using 3D Spheroid Cultures; an in Vivo Relevant Assay Platform

Principal Investigator: Esther A. Guzmán, PhD

Organization: Florida Atlantic University

Grant Progress Report: The main objective of the current project is to identify marine natural compounds with the ability to induce apoptosis in triple negative breast cancer cells grown as spheroids. Cancer cells grown as spheroids better mimic tumors, and thus represent a more clinically relevant way to screen for potential new cancer treatments. While COVID-19 pandemic shutdowns caused delays; the project has made significant progress. Specific aim 1, which consists of screening 3,000 samples from a library or mixed fractions obtained from marine organisms is about 90% complete. The second specific aim has been initiated. More significantly, five pure marine natural compounds with the ability to induce apoptosis in triple negative breast cancer spheroids have been identified thus far. The purification of other leads continues, presenting the opportunity that other compounds, perhaps more potent, will be identified. Studies on understanding how these compounds work (specific aim 3) will start shortly as one compound has activity on both cell lines and appears to be more potent than previous leads. Triple negative breast cancer comprises 10-20% of all breast cancers, but these cancers are among the most aggressive and most lethal. There are no targeted therapies for triple negative breast cancer. Therefore, the compounds identified through this grant have high potential to make a difference in patients with this aggressive form of breast cancer.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

2. Grant #: 9BC03 Impact of Alcohol on Cancer Comorbidities

Principal Investigator: Jennifer L. Steiner, MS, PhD

Organization: Florida State University

Grant Progress Report: Colorectal Cancer (CRC) is among the most prevalent cancers and is the second leading cause of cancer related death, while frequent drinking of moderate to high levels of alcohol increases cancer risk. Cancer cachexia is present in ~50% of colon cancer patients and is characterized by the loss of skeletal muscle and fat mass which directly contributes to decreased muscle strength, quality of life, and treatment compliance and efficacy, as well as increased mortality. Lifestyle factors including alcohol intake, as well as treatments like chemotherapy, may worsen the development of cancer cachexia. The purpose of this project is to determine the impact of alcohol intake on cachexia development as well as the molecular changes incurred by either the prior and/or continued intake of alcohol at tumor initiation. An additional aspect of this work is to investigate the functional impact alcohol may have on skeletal muscle performance in animals suffering from cancer cachexia as muscle weakness can greatly decrease quality of life. These research questions are currently being

addressed using a mouse model of cancer cachexia in which colon cancer cells are implanted into the animal and cachexia develops over the subsequent weeks as the tumor grows. Two different models of alcohol consumption are currently under investigation to determine whether the cachectic effects differ if the patient stops drinking alcohol at the time, the patients get cancer versus continuing to drink. In this first year and following the purchase/setup/trial of all required equipment, the first Aim of the project has begun which will assess alcohol's impact on cachexia development. The team has completed the first block of animals for Aim 1 and are completing the tissue analysis now. Thus far, the data has shown that alcohol led to greater losses in adipose tissue and skeletal muscle mass, especially in the larger leg muscles like the quadriceps (thigh muscle) and gastrocnemius (calf muscle). Interestingly, female mice experienced smaller losses in muscle mass than the males, despite having similar sized tumors, showing females may experience less severe or slower progressing cachexia in this model. Molecular analysis of the muscle tissues has just begun but initial analyses show greater changes in the alcohol-cancer groups, especially in a gene that regulates the loss of muscle mass. Similar to the smaller changes in muscle mass, female mice also show an attenuated molecular response to the cancer and alcohol treatment. These potential sex differences will require additional work in the future to determine if this translates to humans and whether the mechanism can be exploited to also protect males. The molecular changes induced by alcohol consumption before and during cancer will continue to be elucidated this year with the hope of identifying a treatment target or mechanism. In the second half of the year the impact of chemotherapy treatment on these outcomes will be assessed as focus shifts from Aim 1 of the project to Aim 2. All this information will help inform Floridians how detrimental alcohol intake may be to the Floridians' health and quality of life after getting a cancer diagnosis.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

3. Grant #: 9BC04 Modeling Paths to Cancer Health Equity

Principal Investigator: George Rust, MD

Organization: Florida State University

Grant Progress Report: The purpose of this project is to understand the mechanisms that drive racial disparities in breast and colorectal cancer deaths. are analyzing and modeling the levels at which racial disparities in cancer are initiated and amplified, to determine how these mechanisms operate differently in different communities. Application of these models will provide a measure of "likely impact" for planned interventions. The rationale or context for this project is that breast cancer and colorectal cancer are screenable, treatable cancers, yet still cause many cancer deaths, disproportionately in the African American community. The greatest opportunity for reducing cancer deaths in the next decade is to move the benefits of early diagnosis and effective treatment into every community. Instead of testing the same intervention across many communities, our team is developing a strategic decision support system that helps each community understand where to target local interventions to achieve the most

strategic impact on cancer outcomes. A full-time biostatistician / computer modeling specialist was hired and built a collaboration between the Florida State University College (FSU) of Medicine and the FSU Department of Statistics, including an endowed professor and the professor's PhD student working with our FSU College of Medicine researchers. Despite the COVID pandemic, our research team has been meeting weekly by videoconference.
Data has been secured from the National Cancer Institute and the Florida Cancer Data System, and have demonstrated the capacity to securely access, use datasets, and conduct analyses on shared, encrypted server files. Our team has completed initial data work and analyses to begin model-building. The team is preparing our first manuscript around a path analysis of mechanisms that account for racial disparities in stage-at-diagnosis, while also pilot-testing alternative analytic approaches (Bayesian analysis and an instrumental variable approach). Working with the Health Equity Research Institute's statewide community advisory board, team is engaging the community at every stage of the research through telephone and videoconference stakeholder interviews and focus groups. Our team has met with the Florida Center for Interactive Media Development (FCIM) at FSU for app/portal development in parallel with our model-building and community engagement. Further development of more detailed design specs and wireframe models will occur once our initial simulation models are tested. The expected impact to Floridians will come from working with community stakeholders to design and disseminate user friendly web-portals or even smart-phone apps to see what levels of interventions would be most impactful in eliminating cancer disparities in communities. In Florida, every day an African American woman dies who would not have died if our research could eliminate the black-white gap in deaths due to breast cancer. Similar racial disparities exist in colorectal cancer. This is a health outcome disparity that can be overcome. Suffering and costs related to excess late-stage cancer in the black community can be reduced. Lives can be saved.

Follow-on Funding: None at the time of reporting

Collaborations: FSU College of Medicine (medical student Gabrielle LeBlanc) and FSU Department of Statistics (PhD student Inkoo Lee)

Journals: None at the time of reporting

Patents: None at the time of reporting

4. Grant #: 9BC07 Therapeutic Strategies for KEAP1/NRF2 Mutant Lung Cancer

Principal Investigator: Gina M. DeNicola, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Grant Progress Report: Health impact to Floridians: Lung cancer is the leading cause of cancer-related death. Mutations in the the Kelch-like ECH-associated protein 1(Keap1)-Nuclear factor erythroid 2-related factor 2 NRF2/KEAP1 circuit are among the most common mutations in lung cancer, are suggested to cause chemo/radio resistance, and are enriched in tumors that fail to respond to targeted therapy. Research project staff are evaluating new therapeutics specifically designed to target NRF2/KEAP1 mutant tumors and determine whether these mutations are broadly associated with responses to all standard treatments, which may lead to better precision medicine.

AIM1: Target NRF2-regulated metabolism for cancer therapy. The goal of this aim was to evaluate therapeutic approaches that target the metabolism of tumors with mutations in NRF2 and KEAP1, which are found in up to 30% of non-small cell lung cancers (NSCLC). Research project staff are evaluating two approaches, one to block the metabolism of a key nutrient, cysteine (approach #1), and one to exploit a NRF2-regulated enzyme, quinone oxidoreductase 1 (NQO1), to selectively kill mutant tumor cells (approach #2). Approach #1 has resulted in a publication describing a combination strategy to induce cytotoxicity in tumors based on NQO1-activatable compounds, in combination with inhibition of antioxidant enzymes that protect against the toxic effects of these compounds. To identify additional antioxidant enzymes that may be more tumor-selective, our staff has performed a Clustered Regularly Interspaced Short Palindromic Repeats Clustered Regularly Interspaced Short Palindromic Repeats Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9) screen and identified two additional targets. Research staff are currently validating these targets and will move experiments into animal models soon. Staff will also test more potent NQO1-activatable compounds to attempt to overcome antioxidant protection. Progress on approach #2 has been limited by potential immunogenicity of our enzyme-based therapy and possible resistance of tumor cysteine pools to serum cysteine depletion. Research staff are testing these possibilities and additional methods to starve tumors of cysteine.

AIM2: Relate NRF2/KEAP1 mutations and pathway activation with therapeutic response. The goal of this aim is to identify the appropriate patient cohorts to study the effect of KEAP1 and NRF2 mutation status on patients' response to chemotherapy, radiation therapy and immunotherapy. Patients were identified by leveraging Moffitt's enterprise wide data warehouse. Chemotherapy response: Cohorts for the analysis of chemotherapy response have been assembled and study Pathologists have completed review of HandE slides. The Formalin Fixed Paraffin Embedded (FFPE) blocks with the best tumor representation have been identified. The Tissue Core team is currently working on DNA extraction from the selected FFPE blocks for further downstream processes. The next steps are to sequence NRF2 and KEAP1 in these tissues and correlate mutation status with chemotherapy response. Radiation response: Patient analyses have been completed and research staff found that NRF2 activation was associated with regional nodal recurrence following radiation. These results suggest that patients with NRF2/KEAP1 mutations are more refractory to radiation, which should be considered in the patients' treatment. Our team is now determining next steps. Immunotherapy response Two separate cohorts of NSCLC patients treated with PD-1 based immunotherapy with pre-treatment tissue available were identified. Further data query to extract info on cancer characteristics, treatment history and survival data is in progress.

Follow-on Funding: None at the time of reporting

Collaborations: Ohio State University, Department of Radiation Oncology, Columbus, Ohio, Dr. Terrence Williams. Dr. Williams developed databases of patients with non-small cell lung cancer treated with radiation and chemoradiation at the Ohio State University and is an expert on DNA repair and DNA damage response. He is analyzing the association between KEAP1/NRF2 mutations and tumor response to radiation. Following the death of our collaborator David Boothman, who provided b-lapachone for our studies and was going to provide us the more potent compound IB-DNQ, our team has established a collaboration with Dr. Paul Hergenrother, Department of Chemistry, University of Illinois at Urbana-Champaign to obtain IB-DNQ.
Journals: Torrente L, Prieto N, Falzone A, Elkins CM, Boothman DA, Haura EB, DeNicola GM. Inhibition of TXNRD or SOD1 overcomes NRF2-mediated resistance to b-Lapachone. Redox Biology. Feb;30:101440. doi: 10.1016/j.redox.2020.101440. PMCID: PMC6997906

Patents: None at the time of reporting

5. Grant #: 9BC08 Donor γδ T-Cell Infusion for Treatment of High-Risk Leukemia

Principal Investigator: Nelli Bejanyan, MD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Grant Progress Report: Hematopoietic cell transplantation (HCT) is the only curative treatment for many patients with acute myeloid leukemia (AML), which is the most common acute leukemia in adults. If leukemia cells remain detectable in the bone marrow after the initial chemotherapy, HCT may clear the leukemia for some time, but recurrence occurs in up to 65% of the patients and 1-year survival drops to only 25%. Immunotherapy with donor lymphocytes administered after HCT increased cures of patients with residual AML detected at HCT. However, some donor lymphocytes, called aβ T-cells, can attack the patient healthy tissues and result in life- threatening graft-versus-host disease (GVHD). Removal of donor aβ T-cells can eliminate the risk of GVHD but preserve the potent anti-leukemia effect of the γδ T-cells. Isolated γδ T-cells hold promise to increase the cures of AML patients who receive HCT for residual leukemia after chemotherapy. γδ T-cells are rare in the blood. Promising results have shown that γδ T-cells circulating in patient blood can be expanded and used to treat various cancers. However, there is little to no experience with γδ T-cell immunotherapy for AML. Our team has engineered artificial antigen presenting cells (APC) that expand γδ T-cells in the laboratory. Our team hypothesized that donor γδ T-cell infusion to patients with post-chemotherapy residual AML can prevent leukemia recurrence after HCT without causing GVHD and improve survival from 25% to 50%. As a progress to date our team was able to validate our work and show that our APCs efficiently expand (633-fold) healthy donor blood γδ T-cells using a good manufacturing practices (GMP) compliant protocol sufficient to achieve cell doses to treat patients on clinical trial. The aβ T-cell contamination was <1%. Our team also found that expanded γδ T-cells are highly effective in killing cancer cells in a laboratory. The team has clinical trial protocol approved by the Moffitt Scientific Review Committee, and the purpose of this project is to: (1) Determine the safety and effectiveness of APC-expanded donor γδ T-cells infused after HCT for treatment of patients with residual AML in a first-in-human phase 1/1b leukemia recurrence prevention trial. Hypothesis: Donor γδ T-cells infused after HCT will reduce leukemia recurrence without increasing the risk of GVHD. Our preclinical compelling results demonstrate that large scale expansion of healthy donor γδ T-cells can be achieved to treat patients with AML. Thus, many Floridians can potentially benefit from such highly innovative anti-leukemia therapy. (2) Our ongoing laboratory work also studies the leukemia cell killing activity of chimeric antigen receptor (CAR)-engineered γδ T-cells. Here our team explore whether the team can enhance γδ T-cells potency by engineering them with a CAR specific for AML cells. Hypothesis: γδ T-cells engineered with a synthetic receptor will be more effective in killing AML leukemia cells than non-engineered γδ T-cells. These experiments are innovative and will direct future development of synthetic CAR γδ T-cells for immunotherapy of AML.

Follow-on Funding: The Moffitt Cancer Immunotherapy IIT Award Grant; γδ T cell expansion for clinical use; Nelli Bejanyan MD; $99,067

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

6. Grant #: 9BC09 Development of novel TAF1 inhibitors as cancer therapeutics

Principal Investigator: Ernst Schonbrunn, PhD

Organization: Moffitt Cancer Center

Grant Progress Report: The Bromodomain-containing Protein TBP-associated factor 1 (TAF1) is an essential subunit of the general transcription machinery. Although deregulation of gene transcription and evolving plasticity are the underlying cause of drug resistance in cancer, TAF1 is a potential target for the development of drugs aimed at uncontrolled gene transcription. To date, only few bromodomain inhibitors of TAF1 have been developed, but biological effects have not been reported, and no TAF1 inhibitor has reached the clinic. Combined, the present knowledge suggests that TAF1 is a promising yet underexplored target for the development of small molecule inhibitors directed at the transcription machinery of cancer cells through an epigenetic mechanism of action. Recently, the team discovered that a clinical inhibitor of the protein kinase Ataxia Telangiectasia and Rad3-related protein (ATR) also selectively inhibits the second bromodomain of TAF1. This is the first identified kinase inhibitor that targets Bromodomains and Extra-Terminal (BET) outside the BET family. The team determined high resolution co-crystal structures of TAF1 liganded with this inhibitor and close analogues, the knowledge of which provides a new structural framework for the rational design of inhibitors with high potency and selectivity for TAF1 and the ability to concurrently inhibit ATR or other PI3K-related kinases. Our preliminary studies in lung and colon cancer cell lines established that TAF1 inhibitors activate p53 and DNA damage response and cell death. The central hypothesis of this proposal is that TAF1 inhibition by small molecules is a viable strategy to alter the transcription machinery of cancer cells, particularly those evading p53-mediated DNA damage response and apoptosis. The scientific premise is the knowledge gap about the efficacy of chemical inhibition of TAF1 alone and in combination with ATR in cancer. Our objectives are the development and in-depth characterization of novel inhibitors that potently inhibit TAF1 and ATR. This proposal integrates research components from structural biology, cancer biology and medicinal chemistry for the development of dual TAF1-ATR inhibitors as cancer drugs. Progress during the past year involves cell biological, chemical and structural studies. Over 50 analogues of parent compound AZD6738 were synthesized and evaluated for binding and inhibitory activity against TAF1 and ATR. Two compounds, TS1-299 and TS1-252, showed a significant increase in selectivity for ATR vs. ATM compared to the parent compound. Sulfonamide (ZS1-295) and sulfonimidamide (ZS1-322) groups for sulfoximine maintained activity against TAF1, providing opportunities towards the design and synthesis of new chemical matter. New inhibitors are currently being developed using a merged scaffold approach which utilizes the previously reported TAF1 inhibitor GNE371 and our newest analogues of AZD6738 to improve binding potential for TAF1. Structural studies using X-ray crystallography and small-angle X-ray scattering demonstrated global conformational changes of the TAF1 tandem bromodomain upon interaction with certain inhibitors.
Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

7. Grant #: 9BC12 Development of Small Molecule Inhibitors of Wnt/β-catenin Transcriptional Activation.

Principal Investigator: Anthony J. Capobianco, PhD

Organization: University of Miami Miller School of Medicine

Grant Progress Report: Our team discovered three potent and selective compounds (BC-57, BC-45 and BC-14) that target the BCL9/β-catenin interface and disrupt Wnt/β-catenin mediated transcriptional activity. Since the last progress report, the three scaffolds, BC-57, BC-14 and BC-45, have been prioritized for further development. During this quarter, our team advance on the validation of the scaffold of lead compound BC-57 through Structure-Activity Relationship (SAR) study and the medicinal chemistry of the lead compounds (BC57, BC14 and BC45). Currently, the team is performing an extensive SAR campaign to generalize the scaffold based on the related core structures and optimizing potency and other critical properties to identify the clinical candidate. The full report documents these experiments and results. At this time, our staff believe our team has optimized the scaffold and are now optimizing potency on target through computer modeling and SAR chemistry.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

8. Grant #: 9BC13 Data-Driven Identification of Novel Precision Drug Combination Therapies for Prostate Cancer

Principal Investigator: Kerry L. Burnstein, PhD

Organization: University of Miami

Grant Progress Report: In Florida in 2020, 13,950 men will be diagnosed with Prostate Cancer (PC) and 2,800 will die of this malignancy, per American Cancer Society estimates. In advanced PC, tumors often develop “resistance” to drugs, leading to incurable cancer growth. Tumors become resistant in ways that vary between patients. Thus, treating aggressive and drug resistant PC requires tailoring therapies specifically to features of individual patients' tumors. Fortunately, huge amounts of molecular, genetic and clinical information (“big data”) on PC from a broad variety of patients exist. Project staff are exploiting these data to identify and prioritize new and existing drugs and drug combinations to more effectively treat PC. Significant progress was made in three major areas: (1) a computational framework for evaluating new therapeutics was developed based on PC tumor data; (2) PC cell lines were analyzed for genetic

characteristics that must be targeted by new therapeutics; (3) new test compounds were identified by screening millions of compounds for decisive properties of known drugs. Data from 501 PC patient tumors and 52 non-cancer prostate samples was acquired and processed using publicly available information. PC-specific gene “signatures” were identified for computational screening against the constellation of drugs and drug combinations. This method produced “modules” that identify classes of genes or proteins that are over-represented in large numbers of patients and are associated with disease state and patient condition. To generate usable information from the vast field of data types, an integrative database was developed to predict and prioritize effective PC therapeutics. For “real world” testing with new drugs and drug combinations, 12 different cell lines (from PC, advanced PC, and non-cancer prostate tissue) were prioritized and validated. RNA was extracted from these samples and analyzed using “RNAseq,” a method that reveals the “deep” genetic characteristics of (and differences between) these cell lines. Initial RNAseq results were compared to the vast array of prostate cancer data from patients (and patterns from computational analyses). These data will determine specifically how the PC cell lines represent the variations of PC in men and to predict the most effective drugs and compounds for individual tumor types. To identify the first test compounds, a computational selection tool was developed: a comprehensive “prioritization table” (based on important features of 21,202 compounds with known implications for PC) was designed to predict a compound’s activity in PC. The prioritization table was used to screen 25,946,988 commercially available compounds and 27 diverse test compounds were selected. Most of these compounds are not currently in clinical trials. However, compounds in clinical trials will also be considered as the compounds may be combined with other drugs in novel ways leading to synergism, where clinical responses from combinations are greater than the sum of the individual drugs. A large amount of data for PC has already been curated and organized and will be made publicly available to benefit the research community and to maximize the project’s impact. The principal investigator and project staff expect this to become an important resource for the PC research community.

Follow-on Funding: None at the time of reporting

Collaborations: Kerry Burnstein, PhD, University of Miami (PI) serves as principal investigator and has more than 15 years of experience in experimental therapeutics for prostate cancer. Dr. Burnstein is responsible for the overall conduct and management of the project including experimental design, ethical conduct of the research, data interpretation and accuracy of data in all presentations, progress reports and publications.

Stephan Schürer, PhD, University of Miami (collaborator) leads the computational discovery aspects of the project. He assists Dr. Burnstein in the management of the project including experimental design, ethical conduct of the research, data interpretation and accuracy of data in all presentations, progress reports and publications.

Vasileos Stathias, PhD, University of Miami (assistant scientist) works closely with Dr.Khurana to guide the development of robust gene expression modules and disease signatures that characterize the prostate cancer patient sub-populations, mapping cell line and tumor models to patient gene expression signatures and to analyze the results. He works with Dr. Martinez to guide drug synergy analyses methodologies

Rimpi Khurana, PhD, University of Miami (post-doc) performs RNAseq processing and analytics including gene set enrichment and network analysis. Dr Khurana executes computational algorithms based on patient RNASeq data to robustly identify gene co-expression networks. Dr. Khurana matches prostate cancer cell lines and tumor models to patient subpopulations and

generates robust gene-expression signatures that are used to prioritize single drugs and drug combinations for screening.

Maria Julia Martinez, PhD, University of Miami (post-doc) Dr. Martinez performs all cell-based studies and conducts tumor analysis.

Benjamin Sherman, University of Miami (lab manager) conducts supply ordering a well as assists Dr. Martinez with cell-based experiments.

Journals: Issa, N. T.; Stathias, V.; Schürer, S.; Dakshanamurthy, S., Machine and deep learning approaches for cancer drug repurposing. Semin Cancer Biol 2020; doi 10.1016/j.semcancer.2019.12.011.

Patents: None at the time of reporting

9. Grant #: 9BC14 Targeting Heme Dependency in Leukemia

Principal Investigator: Hong Yuan (Rays) Jiang, PhD

Organization: University of South Florida

Grant Progress Report: Iron is essential for cell growth and replication; aggressive or metastasized cancers often rely on large amount of iron intake, named iron-addiction. Targeting cancer iron-addiction represents an attractive novel approach of exploiting cancer metabolic vulnerabilities. However, neither the biochemical mechanisms nor the microenvironment supporting iron-addiction are understood in cancers. In this project, our team plans to use our newly developed single-cell omics technologies coupled with biochemical analyses to pinpoint the biochemical targets in cancer metabolism, specifically in Acute Myeloid Leukemia's (AML). Iron metabolism is tightly linked to heme production, as most iron exists caged in a porphyrin ring forming heme, a protein prosthetic group. Our project has indicated that iron-addiction is inextricably linked to heme overdrive, the heightened embryonic-like heme metabolism, in cancer. Significantly, pancreatic, lung, skin and blood cancers developed iron-addiction and heme-overdrive, as shown by our analysis of genome-wide Clustered Regularly Interspaced Short Palindromic Repeats **(**CRISPR)-based studies. The PI has extensive experience in cutting-edge genomics technologies, combined with expertise from iron and heme biological chemistry (Ferreira) and cancer biology (Reuther and Sebti), our team has made the following keys steps toward targeting cancer metabolic vulnerability in cancer iron addiction. Our team has found, through large scale CRISPR knock out data mining and redox biochemistry analysis, that heme is important for a specialized type of cell death termed ferroptosis. (Manuscript in preparation) The team has established an iron addiction model with two erythroleukemia cell lines both addicted to iron, but with distinct death pathways and resistance mechanisms. Also, the team has generated CRISPR-KO based isogenic line to validate this model. The team has delineated a set of chemo-modulators to precisely probe and intervene cancer iron addiction pathways. Our team has devised a novel intervention strategy to sensitize iron-addicted cancers for metabolic death. Our team is testing this metabolic engineering approach to 'engineer iron-addiction death', and the team is preparing to write a manuscript and patent applications. During this period, our team has trained graduate students and junior researchers in cutting-edge technologies and life science lab operational skills. Our team has established a collaborative network with four partners in three institutions. Our team also presented the work at international and national meetings with acknowledgment to the Florida state funding.

Follow-on Funding: None at the time of reporting

Collaborations: This project is a collaborative research effort of four teams, i.e., Jiang Lab (Genomics, University of South Florida), Ferreira (Biochemistry, University of South Florida) and Reuther (Cancer Biology, MOFFIT cancer research institute), in consultation with Sebti lab (Virginia Commonwealth University, Richmond, VA).

Dr. Ferreira (USF) has close collaborations with Dr. Jiang, and this work would not be possible without the weekly joint lab meetings and very frequent discussions. Dr. Ferreira's biochemistry expertise and in-depth knowledge in iron and heme metabolisms are essential for the carrying out the project. Dr. Ferreira's general research area relates to enzymology and protein chemistry. Dr. Ferreira's research on heme and iron metabolism is internationally recognized.

Dr. Reuther's (MOFFIT Cancer Center) interest in cell signaling pathways, particularly in the field of myeloid leukemia, stems from Dr. Reuther's graduate studies at Duke University where he investigated signaling properties of the leukemic BCR-ABL tyrosine kinase and Dr. Reuther's post- doctoral studies at the University of North Carolina at Chapel Hill. There Dr. Reuther identified and characterized novel oncogenes in acute myeloid leukemia. Dr. Reuther's laboratory at MOFFIT has also studied mechanistic aspects of neoplastic JAK activation, signaling, and therapeutic intervention.

Dr. Sebti (VCU) is a recognized cancer researcher that holds the NCI Outstanding Research award. Dr. Sebti has an excellent working relationship and recently co-published a Nature Communication article on cancer drug discovery with the P.I. This BHC project benefits from Dr. Sebti's rich drug discovery experience and advice. Dr. Sebti will contribute to experimental design, results interpretation and manuscript preparation. Dr. Sebti also is a close colleague of Dr. Reuther.

Journals: None at the time of reporting

Patents: None at the time of reporting

Bankhead-Coley Cancer Biomedical Research Program
Appendix C
Fiscal Year 2019-2020 Active Grants
Funding Fiscal Year 2017-2018

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
8BC01	Mayo Clinic	John Copland, PhD	$815,283	$610,361.00	$204,922.00	5/01/2018	4/30/2021	No	No	No
8BC03	H. Lee Moffitt Cancer Center	Keiran Smalley,PhD	$815,289	$611,460.00	$203,823.00	4/02/2018	3/31/2021	No	No	No
8BC04	H. Lee Moffitt Cancer Center	Robert Gillies, PhD	$815,283	$611,460.00	$203,823.00	3/21/2018	3/31/2021	No	Yes	No
8BC05	University of Central Florida	Otto Phanstiel, PhD	$815,283	$611,462.25	$203,820.75	5/30/2018	3/31/2021	Yes	No	Pending*
8BC06	University of Miami	Xiangxi Xu, PhD	$815,283	$611,460.00	$203,823.00	4/20/2018	3/31/2021	No	No	Yes
8BC07	University of Miami	Sabita Roy, PhD	$815,282	$611,460.00	$203,822.00	4/04/2018	3/31/2021	No	No	Pending*
8BC09	University of Miami	Eric Weider, PhD	$1,358,805.00	$1,262,664.00	$96,141.00	6/08/2018	3/31/2021	No	No	No
8BC10	University of Miami	Shanta Dhar, PhD	$815,283	$611,462.25	$263,820.75	4/11/2018	3/31/2021	No	Yes	No

*Received notification of follow-on funding but award has not been finalized.

1. **Grant #:** 8BC01 Novel Metabolic Target Induces Immunogenicity and Antitumor Synergy with Immune Checkpoint Inhibitor Leading to Survival Benefit

   Principal Investigator: John A. Copland, PhD

   Organization: Mayo Clinic

   Grant Progress Report: This project was funded in April 2018. In this grant, our team propose to demonstrate antitumor synergy and survival benefit using SSI-4, a novel therapy developed in the Copland laboratory, in combination with anti-Program Death (PD)-1 or anti-PD- L1 antibody (immune checkpoint inhibitors) against Triple Negative (TNBC) and HER2+ Breast Cancers as well as colon cancer and melanoma. Our preliminary data show that combined SSI-4, a novel inhibitor of fatty acid metabolism targeting a protein called Stearoyl CoA Desaturase 1 (SCD1), with anti-PD1 therapy antagonizes tumor growth in mouse breast cancer models as well in melanoma. Our SSI-4 compound has broad activity and inhibits growth across all cancers. Our work should lead to clinical trials in multiple cancers with expectations to increase survival for patients diagnosed with breast cancer, colon cancer and/or melanoma. Our team also propose to study the mechanism by which SSI-4 sensitizes the tumor to the immune checkpoint inhibitor.

   Additionally, the team is examining a novel mechanism of action. Blocking SCD1 with SSI-4 leads to tumor cell death and in the process may cause a protein called calreticulin (CRT) to go to the cell membrane. When CRT is on the cell membrane, it has potential to activate immune cells that can then attack and kill tumor cells. Thus, the team has performed experiments that show CRT is on the cell membrane when tumors are treated with SSI-4 alone and with SSI-4 plus anti-PD-1 antibody. Our team have completed this in vitro and in vivo. Thus, this therapy uses two mechanisms to kill tumor cells that include 1) a direct effect of inducing endoplasmic reticulum (ER) stress by blocking SCD1 using SSI-4 leading to tumor death as well as 2) activating the immune system by making the tumor cells recognized by immune cells (macrophages, T, NK cells) to attack and engulf tumor cells. The team has several experiments underway including tumor cells where our team has used cutting edge technology to silence CRT. In cells silenced for CRT, the team would not expect to see activation of the immune system and synergy with SSI-4 plus anti-PD-1 antibody. The team is excited to start these experiments now that our team has created these unique breast cancer cell lines silenced for CRT expression.

   Follow-on Funding: None at the time of reporting

   Collaborations: None at the time of reporting

   Journals: None at the time of reporting

   Patents: None at the time of reporting

2. **Grant #:** 8BC03 Defining and Therapeutically Targeting HDAC8-Driven Reprogramming in Melanoma Brain Metastasis Development

   Principal Investigator: Keiran Smalley, PhD.

   Organization: H. Lee Moffitt Cancer Center and Research Institute

Grant Progress Report: Among all tumor types, melanoma has a high propensity to metastasize to the brain. Brain involvement is clinically evident in ~30% of melanoma patients (as high as 75% at autopsy) and the brain is often the major site of disease progression; even when extracranial disease is well controlled. Little is currently understood about the biology of melanoma brain metastasis and this represents a major knowledge gap that limits our ability to deliver long-term therapeutic responses to melanoma patients. In our initial studies, our team uncovered a novel gene expression program regulated through an enzyme called Histone DeACetylase (HDAC)-8 that reprogrammed melanoma cells to form brain metastases in animal models. The goal of this project is to define the gene expression program that is controlled by HDAC8 and our team will determine how this cellular state permits the melanoma cells to form new tumors in the brain. In the past year our team has made major progress in understanding how HDAC8 drives the metastatic program. The team identified a novel mechanism whereby HDAC8 directly regulated the acetylation of the transcription factor c-JUN, leading to an increase in its activity and the increased expression of genes that allowed melanoma cells to escape BRAF inhibitor therapy, survive in the circulation, invade into the lung and form new metastases. One mechanism of HDAC8-driven metastasis involved non-canonical ephrin type-A receptor 2 (EphA2) signaling, which permitted the melanoma cells to adopt an amoeboid phenotype and migrate through endothelial cell layers into new organs. Part of this work was published in the journal Cancer Research, with another study currently being revised for the Journal of Investigative Dermatology. To study the entire process of metastasis from early tumor development through egress from the primary tumor to new organs, our team developed a new mouse model of BRAF-mutant/PTEN-silenced melanoma with increased HDAC8 expression. It was found that induction of HDAC8 in this mouse model increased tumor development, leading to shorter survival times. Our team is currently performing long term follow up studies to determine if there is an increase in the incidence of brain metastasis development. A preliminary analysis of the immune environment of these HDAC8- driven tumors has demonstrated an alteration in immune cell infiltrate, with fewer CD4+ and CD8+ T-cells being identified. Our team, therefore, expect that part of the effects of HDAC8 introduction may be mediated through immune escape. In other studies, our team performed unbiased Clustered Regularly Interspaced Short Palindromic Repeats **(**CRISPR/Cas9) screens to identify genes that are suppress brain metastasis development. Working with Dr. Licht's lab at the University of Florida the team transduced melanoma cells with guide RNAs for >200 epigenetic regulators and CAS9 and then injected melanoma cells intracardiac. Resulting brain metastases were extracted and sequenced for guide RNA dropout. These studies identified the RNA acetyltransferase NAT10 as being a key negative regulator of brain metastasis development. There is evidence from our proteomic analysis of HDAC8 that NAT10 interacts with HDAC8 and that HDAC8 regulates NAT10 function through de-acetylation at Lys 284. Our team is currently exploring how HDAC8 regulates NAT10 to regulate brain metastasis development.

Follow-on Funding: None at the time of reporting

Collaborations: This project is collaboration between the labs of Dr. Keiran Smalley at Moffitt Cancer Center and Dr. Jonathan Licht at the University of Florida Cancer Center. The proposed work leverages the unique experience of Dr. Smalley in melanoma and brain metastasis biology and Dr. Licht in epigenetic regulation and CRISPR screening. Dr. Licht and Dr. Smalley's lab have initiated regular ZOOM video conferencing meetings that are held every 3 weeks. Drs. Licht and Smalley met at the University of Florida to discuss this project in December 2019, and the doctors' labs met in Orlando for a one-day scientific retreat on January 25th, 2020. One graduate student Chao Zhang (Moffitt Cancer Center/University of South Florida) is receiving training under this award. Other people performing research on this award are Dr. Michael Emmons (Staff Scientist, Moffitt), Dr. Amin Sobh (Post- doc, University of Florida) and Dr.

Richard Bennett (Research Assistant Professor, University of Florida).

Journals: Emmons, M.F., Faião-Flores, F., Sharma, R., Thapa, R., Messina, J.L., Becker, J.C., Schadendorf, D., Seto, E., Sondak, V.K., Koomen,

J.M., Chen, Y.A., Lau, E.K., Wan. L., Licht, J.D., Smalley, K.S.M.:
HDAC8 regulates a stress response pathway in melanoma to mediate escape from BRAF inhibitor therapy. Cancer Res. 79(11):2947-2961, 2019. PMID: 30987999

Zhang C, Smalley, I., Emmons, M.F., Sharma, R., Izumi, V., Messina, J.L., Koomen, J.M., Pasquale, E.B., Forsyth, P.A., Smalley, K.S.M.: Non-canonical EphA2 signaling is a driver of tumor-endothelial cell interactions and metastatic dissemination in BRAF inhibitor resistant melanoma. Journal of Investigative Dermatology. In revision.

Patents: None at the time of reporting

3. **Grant #:** 8BC04 Targeting the Lipogenic Phenotype Induced by Extracellular Acidosis in Breast Cancer.

Principal Investigator: Robert J Gillies, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Grant Progress Report: Malignant tumors exhibit altered metabolism resulting in a highly acidic extracellular microenvironment. Adaptation to acidic conditions is a pre-requisite for tumor cells to survive, thrive, and out-compete the stroma into which they invade. Here our team showe d that these adaptation mechanisms include the accumulation of cytoplasmic lipid droplets (adiposomes); dynamic organelles that store neutral lipids surrounded by a shell containing perilipin (PLIN2) proteins. Our studies revealed that PLIN2 is strongly associated with poor overall survival in breast cancer patients. Our team showed that the source of adiposomal lipids is de-novo and endogenous. MS/MS of 13C tracers showed that source of the lipid precursors is primarily autophagy- derived ketogenic amino acids. Acidosis induced adiposomogenesis was attenuated when cells were treated with fatty acid synthesis inhibitors such as TOFA or C75. Further, these inhibitors were selectively cytotoxic under acidic conditions indicating that adiposomogenesis is a survival mechanism. Accumulation of adiposomes is triggered by activation of the acid sensing Gq-coupled receptor, Ovarian cancer G-protein-coupled-receptor-1 (OGR1) whose signal is transduced via pAkt and is over expressed in breast tumors. Moreover, our studies revealed that Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9) mediated OGR1 depletion in breast cancer cell lines inhibited acid-induced adiposome formation, inhibited autophagy, affected cell viability in vitro, and tumor growth in vivo in xenograft models. Hence, our team identified that adiposomogenesis is a highly regulated process related to storing autophagic products, which is important in cell survival in acid stress. This increased dependence on lipid metabolism has revealed novel therapeutic vulnerabilities and identified OGR1 signaling as a clinically relevant therapeutic targets for breast cancer. Data collected so far from this project is compiled into a manuscript titled' Lipoogenesis is a key metabolic adaptation to acid stress' and is now under external peer review in JCI (Journal of Clinical Investigations). In addition, an e-poster titled 'Causes and consequences of adiposomogenesis in breast cancer cells' was presented at AACR annual meeting 2020.

Follow-on Funding: None at the time of reporting

Collaborations: The University of Florida, Department of Pathology, Immunology and Laboratory Medicine in Gainesville, FL has been involved in this research project through analytical measurement of adiposomal lipid species using ultra-high-pressure liquid chromatography (UHPLC) coupled to high resolution mass spectrometry. Currently, Dr. Timothy Garrett (SECIM, University of Florida) and his postdoctoral fellow, Dr. Iqbal Mahmud have been working on analytical improvement of methodologies and helped to utilize new software tools to better understand the isotopic flux of precursors into intact lipids species. In addition, our team collaborate with Dr. Matthew Merritt, the Department of Biochemistry and Molecular Biology at University of Florida on Nuclear Magnetic Resonance (NMR) analysis of adiposomal lipids.

Journals: Manuscript titled 'Lipoogenesis is a key metabolic adaptation to acid stress' is now under external peer review in JCI (Journal of Clinical Investigations). Authors: Smitha Pillai, Iqbal Mahmud, Michael Langsen, Jonathan W. Wojtkowiak, Jonathan Nguyen, Marilyn Bui, Robert Gatenby, Timothy Garrett and Robert Gillies.

Poster entitled "Causes and consequences of adiposomogenesis in breast cancer cells' Authors: Smitha Pillai, Iqbal Mahmud, Michael Langsen, Jonathan W. Wojtkowiak, Jonathan Nguyen, Marilyn Bui, Robert Gatenby, Timothy Garrett and Robert Gillies was presented on June 22-24, at AACR virtual annual meeting II as part of AACR annual meeting 2020.

Patents: None at the time of reporting

4. **Grant #:** 8BC05 Developing Polyamine Transport Inhibitors for the Treatment of Human Cancers

   Principal Investigator: Otto Phanstiel, PhD

   Organization: University of Central Florida

   Grant Progress Report: Synthesize non-polyamine containing polyamine transport inhibitors (PT/s). We have made significant progress in optimizing our non-polyamine containing PTI designs. In our October 2019 report, we described our efforts to improve upon the lead UCF420 design and here we describe our efforts to further explore this promising design via new derivatives. In an effort to understand polyamine metabolism in pancreatic cancers, a systemic study was conducted wherein relative mRNA expression patterns were compared in early PanIN-1 lesions vs PDAC tumors and stroma. From this preliminary study, SLC12A8, a known cation/chloride cotransporter, was identified as another putative polyamine transporter. 1 In contrast to ATP13A3, the SLC12A8 gene was found to be significantly overexpressed (high mRNA) in stroma compared to PDAC tumors. This insight formulated our polyamine exchange hypothesis where stromal cells use one polyamine transporter (SLC12A8) to import low value polyamines (e.g., putrescine) and tumor cells use a different transporter (ATP13A3) to harvest high value polyamines (spermidine) from the extracellular space. Therefore, it was important to further examine the expression and localization of this protein in different human pancreatic cancer cell lines. Since our hypothesis was that SLC12A8 operates in the stroma and ATP13A3 operates in tumor cells, we needed to demonstrate that SLC12A8 was either not expressed, had a non-functional isoform, or did not respond to polyamine stimuli in tumor cells. This effort was important as we needed to understand whether the tumor cell SLC12A8 protein could respond to exogenous polyamine stimuli by changing its intracellular localization (i.e. move to the plasma membrane) as seen with the ATP13A3 protein (see July 2019 report). Since commercially available antibodies against SLC12A8 have been discontinued, we purchased a customized SLC12A8 antibody from GenScript.

Follow-on Funding: None at the time of reporting

Collaborations: The University of Central Florida (UCF) is the primary site of research for this project along with Advent Health (AH) as a secondary site. UCF students will work on Aims 1-3 with AH participating in some aspects of Aim 3. Currently, there are four students working on this project, namely Ms. Aiste Dobrovolskaite (PhD student, Phanstiel lab), Ms. Sai Preethi Nakkina (PhD student, Altomare lab), Mukund Tantak (postdoctoral research associate, Phanstiel lab) and Dr. Vandana Sekhar (postdoctoral research associate, Phanstiel lab).

Journals: Identification of Trypanosoma cruzi Polyamine Transport Inhibitors by Computational Drug Repurposing. Chantal Reigada, Melisa Saye, Otto Phanstlel IV, Edward Valera-Vera, Mariana Renee Miranda, Claudio A. Pereira. Front. Med. 2019, 6, No. 256, Nov 8, 2019, https://doi.org/10.3389/fmed.2019.00256

Polyamine Blocking Therapy Decreases Survival of Tumor-Infiltrating lmmunosuppressive Myeloid Cells and Enhances the Anti-Tumor Efficacy of PD-1 Blockade. Susan K. Gilmour, Eric T Alexander, Kelsey Mariner, Julia Donnelly, and Otto Phanstiel. Molecular Cancer Therapeutics, 2019, submitted manuscript MCT-19-1116.

The involvement of polyamine uptake and synthesis pathways in the proliferation of neonatal astrocytes. Christian J. Malpica-Nieves, David E. Rivera-Aponte,Flavia A. Tejeda-Bayron ,Angel M. Mayor,Otto Phanstiel, RObiger W.Veh, Misty J. Eaton, Serguei N. Skatchkov.Journal of Developmental Biology, submitted manuscript November 21, 2019, Manuscript ID: jdb-661903. Rejected and then resubmitted to Biomolecules on Jan 15, 2020.

Patents: None at the time of reporting

5. **Grant #:** 8BC06 Therapeutic Targeting of RAGE in Breast Cancer Progression and Metastasis

Principal Investigator: Barry Hudson, PhD to Xiangxi Xu, PhD

Organization: University of Miami

Grant Progress Report: In the current funding period, regardless of the major restriction in place due to the COVID19 lockdown, the team has made major progress with testing our RAGE inhibitors in breast cancer cell models and in animal models of breast cancer. In cell assays, our team has further performed functional assays that show RAGE inhibitors impair metastatic function of breast cancer cells. Further, the team has made new discoveries with respect to the amount of drug required to impar metastasis with RAGE inhibitors in animal models and their impact on tumor cell growth and progression.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

6. **Grant #:** 8BC07 Role of Microbiome in Modulating Liver Metastases in Colon Cancer

   Principal Investigator: Ashok Saluja, PhD to Sabita Roy, PhD

   Organization: University of Miami Miller School of Medicine

   Grant Progress Report: Colon cancer is the second most common cause of cancer related deaths in United States. Every year over 50,000 people die due to this disease. Most of these deaths are due to metastases. Our team has hypothesized that exposure to gut microbiome induces immunosuppression and creates a permissive environment for the growth of colon cancer metastases. This hypothesis will be tested through following three aims: To Elucidate the Role of Gut Microbiome in Modulating Liver Metastases. To Evaluate the Role of Immune Modulation in Gut Microbiome Induced Promotion of Liver Metastases. To Elucidate the Role of Toll-Like-Receptors in Gut Microbiome induced Enhancement of Liver Metastases.

   Our team has focused on narrowing down the role of gut bacterial species that translocate from the gut to the site of tumor (liver) and promote tumorigenesis. By using 16S Bacterial DNA Sequencing on the fecal samples collected from mice that were treated with the antibiotic cocktail vs saline, our team observed that the bacterial taxa present in metastatic livers bore close resemblance to gut bacteria thereby confirming the translocation theory. Our team has also shown that out of all the components of antibiotic cocktails (Vancomycin + Neomycin + amphotericin B + metronidazole + ampicillin) administered to mice for modulating the gut microbiome, Vancomycin alone has an impact on the translocation of gut microbiota from gut to liver. Regarding the role of Toll- like receptors, TLR2 is a gram-positive sensor present on antigen presenting cells and our results, along with results reported in previous quarterly reports, strengthens our assertion that distinct gram-positive ligands promote tumorigenesis by stimulating TLR2. Additionally, the 16S Sequencing also revealed that the bacterial taxa which were associated with increased tumor burden. Since the last reporting period, the team has finally secured an IRB approval to recruit patients with colorectal and pancreatic cancer and collect stool samples at various stages of the patients' treatment which would be sequenced to identify the change in microbial species. The IRB was approved in November 2019 and the team is happy to report that our staff have collected stool samples from seven cancer patients and two volunteers so far and experiments from eight subjects are in late stages of data collection. Since Covid-19 the team has been unable to recruit more patients. Our team hopes to continue these studies once normal operations resume on campus.

   Follow-on Funding: Florida Department of Health Grant Mechanism; Role of Microbiome in Modulating Liver Metastasis in Colon Cancer; Sabita Roy PhD; Pending

   Collaborations: None at the time of reporting

   Journals: None at the time of reporting

   Patents: None at the time of reporting

7. **Grant #:** 8BC09 Multiplex Imaging Resource for State of Florida

   Principal Investigator: Eric D Wieder, PhD

Organization: University of Miami

Grant Progress Report: The purpose of this project is to establish a new Imaging Center at Sylvester Comprehensive Cancer Center (SCCC) to enhance cancer research at SCCC and for other cancer researchers in Florida using a technique called mass imaging cytometry (MIC). Historically, there are various staining methods which allow pathology labs to identify various characteristics of tumors from a patient biopsy, but a more sophisticated way uses antibodies tagged with colors to be able to distinguish different markers on cells within the tumor. In most labs, it is typical to be able to look at 1-4 markers at the same time, although there is specialized equipment that can look at 10-12 at a time. The newly implemented MIC facility uses metal atoms instead of colors to tag and identify each marker, which has increased the number of markers that can be studied simultaneously to 30 markers or more. This disruptive technology has begun to be used by scientists all over the world and results are beginning to be published. This facility will allow researchers at SCCC and in Florida to stay competitive in the developing areas of cancer research since it is becoming more common that these complex measurements need to be included in any study that involves either heterogeneity of tumors (differences within them), or immune therapy. Progress in the past year: The MIC facility was presented as a developing service line within the Flow Cytometry Shared Resource in our National Cancer Institute (NCI) Cancer Center Support Grant (CCSG) application. The MIC was viewed very favorably during the review and Sylvester became an NCI-designated cancer center in July 2019. SCCC recognized that the barrier to entry to use the equipment due to cost of reagents is high, therefore a pilot grant program was created using SCCC funds to award five researchers $10,000 each to perform pilot studies using MIC. Thirteen innovative proposals were received and five of them were funded including studies of brain, blood (2), pancreas, and colon cancer. Those studies were initiated, which will result in pilot data to leverage additional grant funding. Furthermore, SCCC established a reagent bank to reduce the cost of reagents for researchers. A data analysis workshop was held to promote use of the equipment (January 7-9) which were well attended (20 attendees total). Finally, our team used Bankhead Coley funds to invest in a new data analysis software package (Visiopharm) and a powerful computer workstation which will facilitate our ability to analyze the complex data generated in this facility. The team lost some momentum in usage of the facility during the COVID-19 pandemic outbreak, but our staff is working to bring the utilization back to prior levels. Impact to Floridians and ROI: Seven researchers at SCCC have performed pilot studies using MIC including one user from Moffitt Cancer Center in Tampa who utilized MIC to study 79 breast cancer patient samples. Our team continues to work other members of the Florida Academic Cancer Centers Association (ACCA) to promote usage for all cancer research in the state.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

8. **Grant #:** 8BC10 Multifunctional Nanoparticle for Targeted Combination Therapy for Prostate Cancer

Principal Investigator: Shanta Dhar, PhD

Organization: University of Miami

Grant Progress Report: During the year two of the grant, our team has focused on synthesizing and troubleshooting the bone metastasis inhibitor, pamidronate prodrug for encapsulation in the NanoParticle (NP). This is an extremely important aspect of the project since the commercially available compounds cannot be incorporated in NPs which are hydrophobic. Over the past few months, our major accomplishments are the team has first optimized and synthesized the PLGA-Pamidronate prodrug molecule as proposed in the grant and successfully optimized multifunctional NPs containing the inhibitor. Our initial studies with the PLGA-Pamidronate encapsulated NPs demonstrated reduction in invasion/migration of metastatic Prostate Cancer (PCa) cells. The multifunctional NPs also demonstrated the ability to inhibit osteoclastogenesis, a process by which osteoclasts are produced from blood cells such as monocytes or macrophages. During the process of the above-mentioned studies, the team realized that the amount of pamidronate loaded in the NP cannot be quantified accurately as the presence of PLGA interferes with available bisphosphonate quantification methods for pamidronate interferes. Pamidronate is an extremely hydrophilic molecule which requires us to make a hydrophobic analog in order to encapsulate into the hydrophobic core of the NP. The hydrophilicity of Pamidronate made standard coupling methods difficult to accomplish. Thus, in the last year, our team has optimized several aspects related to solubility and how to conduct chemical reactions using pamidronate. Our team was able to successfully synthesize a hydrophobic analog which can be incorporated in the core of the NPs. The team was also able to quantify the pamidronate analogue using High Performance Liquid Chromatography (HPLC). However, the yield of the is product is low and the team believes that the inefficiency of this technique can be a problem for potential clinical translation of this work and thus the team set out to develop another version of pamidronate prodrug. In the metabolic front of PCa, the team found that androgen promotes the PCa cells towards more fatty acid oxidation pathway. Our team has not faced any major challenges related to shared resources and institutional commitments. The scientific aims of the proposed research remain the same and our team is making progress in achieving the goals mentioned in the grant application. In the coming year, our team will continue to work on prostate cancer metastasis inhibition using our platform, understand the metabolic pattern of prostate cancer cells when treated with the multifunctional nanoparticle with or without radiation and understand the relation between resistance and metabolic changes. Our team will continue to work on establishing the Patient Derived Xenografts (PDX) tumor for studying this platform in vivo. In the coming year, our main objectives will be to study therapeutic efficacy of the system in PDX in vivo model when combined with radiation. The team will also focus on understanding the efficacy of this multifunctional nanoparticle platform on cancer stem cells. Beyond the grant period, the team will continue to work on this project and include an additional targeting aspect since the team discovered that metabolism plays significant role towards resistance development, our team will be delivering the combined therapy selectively inside the mitochondria of PCa cells.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: Metabolic Modulation of the Tumor Microenvironment Leads to Multiple Checkpoint Inhibition and Immune Cell Infiltration. Kolb, David; Kolishetti, Nagesh; Sarkar, Shrita; Surnar, Bapurao; Guin, Subham; Shah, Anuj; Dhar, Shanta. ACS Nano. December 2019.

Patents: None at the time of report

Bankhead-Coley Cancer Biomedical Research Program
Appendix D
Fiscal Year 2019-2020 Active Grants
Funding Fiscal Year 2016-2017

Grant #	Organization	Principal Investigator	Award Amount	Life To Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
7BC02	University of Florida	Andrew Judge, PhD	$ 1,226,836	$ 1,158,677.50	$ 68,158.50	3/22/2017	2/29/2020	No	Yes	No
7BC03	University of Miami	Emmanuel Thomas, PhD, FAASLD	$ 1,866,436	$ 1,244,290.00	$ 622,146.00	3/17/2017	2/28/2022	No	Yes	No
7BC04	H. Lee Moffitt Cancer Center	Clement K. Gwede, PhD, MPH, RN, FAAN	$ 828,125	$ 552,080.00	$ 276,045.00	3/15/2017	2/28/2022	No	No	Yes
7BC06	Florida Atlantic University	Amy E. Wright, PhD	$ 622,683	$ 518,902.50	$ 103,780.50	3/27/2017	2/29/2020	No	Yes	Yes

1. **Grant #:** 7BC02 Initiating Mechanisms of Cancer Cachexia

   Principal Investigator: Andrew R Judge, PhD

   Organization: University of Florida

   Grant Progress Report: The research team found that various cachexia inducing tumor cells, including primary human pancreatic cancer cells, release IL- 8 (CXCL8) and/or CXCL1. Human pancreatic Tumor Associated Stromal (TAS) cells also release IL-8 and CXCL1 and given that cancer cells and TAS cells collaborate within the tumor microenvironment our team further measured both IL-8 and CXCL1 release from cancer cell/TAS cell co-cultures. This identified a synergistic increase in IL-8 but not CXCL1, and this was due to cancer cells stimulating greater release from TAS cells. The team has also identified that serum levels of both IL-8 and CXCL1 are increased in cachectic pancreatic cancer patients compared to non-cancer control patients. In agreement with this, a recently published study demonstrated that IL-8 is significantly increased in cachectic compared to non-cachectic pancreatic cancer patients when all patients were considered as a whole, and when resected patients were separately considered or when locally advanced patients were separately considered. Further, the same study showed that IL-8 positively correlated with body weight loss and negatively correlated with muscle mass measured from CT scans and concluded that IL-8 is a characteristic of pancreatic cancer cachexia. Our mechanistic work has significantly expanded these clinical findings. Indeed, treatment of muscle cells (myotubes), in vitro, with IL-8 or CXCL1 induces significant atrophy. This myotube atrophy is mediated through the CXCR2 receptor and ERK1/2 activation since treatment with a CXCR2 antagonist or an ERK1/2 inhibitor prevents IL-8 and CXCL1 induced wasting. Further, our team has found that myotube atrophy in response to conditioned media from human pancreatic cancer cells is significantly attenuated with neutralization of IL-8 or CXCL1, or with inhibition of CXCR2 or ERK1/2. The team transitioned these experiments to mice and found that injection of recombinant IL-8 or recombinant CXCL1, intra peritoneal (IP) into mice induces significant skeletal muscle wasting. The team has further identified that global deletion of CXCR2 is protective against pancreatic cancer-induced cachexia. Indeed, tibialis anterior, gastrocnemius, soleus, and gonadal fat mass were each spared in tumor bearing Cxcr2fl/fl-Cre+ mice compared to Cxcr2fl/fl-Cre- mice. Since Ly6G+ cells are the most prominent source of CXCR2 in mice our team also treated pancreatic tumor bearing mice with an anti-Ly6G antibody, or an isotype control. Here our team found that tumor bearing mice treated with the anti-Ly6G antibody were protected from cachexia, compared to the tumor bearing isotype control group. Thus, depletion of Ly6G+ cells, the dominant cell type expressing CXCR2, has a similar effect to Cxcr2 deletion in protecting against cachexia. Our team next determined whether pharmacological inhibition of CXCR2 signaling could inhibit cachexia by treating pancreatic tumor bearing mice with the CXCR2 antagonist, SB225002 (the same inhibitor used in our in vitro experiments). In these experiments our team found that tumor bearing mice treated with SB225002 were spared from muscle wasting, but not fat wasting, compared to vehicle treated tumor bearing mice. Based on these findings, and the knowledge that skeletal muscle also expresses CXCR2, the team subsequently knocked down CXCR2, using a shRNA construct, in the skeletal muscle of mice injected orthotopically with pancreatic cancer cells, and found that this abolished the tumor-induced skeletal muscle wasting. These findings suggest that CXCR2 in skeletal muscle is required for the normal muscle atrophy that occurs in response to pancreatic tumors. Since muscle tissue contains multiple cell types, the team next questioned whether CXCR2 expression in skeletal muscle cells is required for cancer cachexia. To do this our team crossed Cxcr2 fl/fl mice with skeletal muscle specific Cre mice (human skeletal actin (HSA)-Cre mice). However, in these experiments our team found

comparable cachexia in wild type and skeletal muscle specific Cxcr2 knockout tumor bearing mice. Thus, overall, these findings demonstrate that CXCR2 is required for pancreatic cancer cachexia, but that CXCR2 expression in skeletal muscle cells is not required. To determine whether secretion of the CXCR2 ligands, IL-8 and CXCL1, from pancreatic cancer cells are individually required for cachexia in response to human pancreatic tumors our team deleted each from L3.6pl cells and injected WT, IL-8-/- or CXCL1-/- cells into the pancreas of mice. The team found that each of these cell lines induced the same degree of cachexia, suggesting that tumor-derived IL-8 alone and CXCL1 alone do not cause cachexia. However, the team did find differences in the serum and tumor profile of cytokines and chemokines, between WT and knock out (KO) cells, and between IL-8 and CXCL1 KO cells. This latter finding suggests that co-deletion of IL-8 and CXCL1 may have additive effects and could attenuate cachexia. Our team is awaiting the completion of these double KO cells to test this question.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: IL-8 released from human pancreatic cancer and tumor- associated stromal cells signals through a CXCR2-ERK1/2 axis to induce muscle atrophy. Chandler S. Callaway1*, Andrea E. Delitto1*, Rohan Patel1, Rachel L. Nosacka1, Andrew C D'Lugos1, Daniel Delitto2, Michael R Deyhle1, Jose G. Trevino2, Sarah M. Judge1 and Andrew R. Judge1 Cancers. Published November 2019.

Patents: None at the time of reporting

2. **Grant #:** 7BC03 Identifying Infection and Molecular Determinants of Health Disparities in HCV Infected Minority Populations for the Prevention of Early Detection of HCC

   Principal Investigator: Emmanuel Thomas, MD, PhD, FAASLD

   Organization: University of Miami

   Grant Progress Report: First, our team would like to thank the Bankhead-Coley program for their support of this work. Our greatest achievement for the last year was having the state of Florida being recognized for its efforts to eliminate HCV (https://www.hhs.gov/hepatitis/get-involved/hepatitis-elimination/index.html). Through this funding, our team is also assessing the impact of COVID-19 on liver disease progression to hepatocellular carcinoma. Overall, the study team is very excited about this project and are confident that our team will achieve the goals of this funded grant. The team has collected clinical information and now have a comprehensive database for 2,080 patients with liver disease that are at increased risk of developing hepatocellular carcinoma (HCC). As described in Aim1 of the grant, the team has completed the cross-sectional analysis that will be carried out now in 2,080 patients to identify novel clinical covariates that may drive liver disease progression. Our goal is to identify covariates that may drive hepatocarcinogenesis in order to identify Floridians who are at risk earlier so that interventions can be employed. Emphasis in our future work will be focused on Floridians with highest and intermediate risk of developing HCC and trying to generate a new risk calculator that incorporates Fibroscan. Toward our initial efforts to develop a liver cancer risk calculator that utilizes race/ethnicity, the team has begun to develop new non-invasive prediction models for fibrosis and cirrhosis. Since cirrhosis is the most powerful predictor for the risk of developing HCC and because liver biopsies are being utilized less by the clinical community, our team

believes these efforts will lay the foundation for our future work. Using multivariable statistical modeling, the team can accurately predict cirrhosis (Metavir F4 fibrosis stage) utilizing noninvasive clinical markers and are now mapping liver disease based on our Zip Code based data. Importantly, our team has submitted follow on grants to the National Institutes of Health (NIH), Department of Defense (DOD) and a recent application for a Bankhead Infrastructure grant that will take our work and expand it to the rest of Florida by leveraging the OneFlorida consortium based at the University of Florida. Furthermore, since starting this Bankhead-Coley Grant, the PI has been awarded a 5-year, renewable grant from the NIH for $1.9 million. This NIH funded study is focused on understanding inflammatory mechanism that lead to chronic viral infections in the liver through basic science laboratory studies. The grant is a nice complement to this clinical study, supported by the Florida Department of Health, and the funding from this grant has increased since the team subsequently received a minority supplement to support a graduate student. In addition, the PI has been awarded a new $300,000 grant from Gilead Sciences (GS) to screen for HCV and HIV in the University of Miami Emergency department. Furthermore, our team recently established the Florida HCV-HCC/Liver Cancer Consortium with Moffitt Cancer Center, University of Florida and Jacksonville Mayo Clinic through three previous meeting. The most recent meeting was held in Orlando on August 16, 2019.

Follow-on Funding: None at the time of reporting

Collaborations: This project is being performed at the University of Miami Miller School of Medicine. Furthermore, our team recently formed the Florida HCV-HCC/Liver Cancer Consortium with Tampa Moffitt Cancer Center (Dr. Anna Giuliano-Center for Infectious Cancers), University of Florida Gainesville (Drs. David Nelson-Hepatology and Betsy Shenkman-Medicine) and Jacksonville Mayo Clinic (Dr. Tushar Patel- Transplant Hepatology) through an initial meeting that was held at Moffitt Cancer Center in Tampa in October 2017. The next meeting was held in Miami on May 7, 2018 and Dr. Emmanuel Thomas co-lead that meeting in Miami. Our team held a meeting at the University of Florida in Gainesville on April 19, 2019 and the next meeting took place in Orlando on August 16, 2019. It is anticipated that additional grants will be submitted with work from this multi-institutional group that is focused on liver cancer/HCC. This project is currently providing training to four University of Miami Graduate students: Alexandra Debose-Scarlet (3rd year medical student-MD program), Jasmine Edwards (3rd year graduate student-PhD program), Owen Willis (2nd year graduate student-PhD program) and Alejandro Badilla (2nd year graduate student-PhD program) and three University of Miami undergraduate students (David Barr, Danae Lally and Shree Patel). In addition, the team has developed and started a new training program for interested medical students at the University of Miami specifically focused on oncology from a multidisciplinary perspective. It includes educational materials and speakers from radiation oncology, surgical oncology, medical oncology, radiology, interventional radiology, pathology, gynecologic oncology and immuno-oncology. This will greatly expand the number of students that will benefit from this state funding that is supporting the PI since our team currently have 26 individuals in our oncology training pathway for medical students.

Journals: Stem Cells and Liver Diseases. Hu Z, Xia Y, Hong SG, Thomas E, Li D. Stem Cells Int. 2019 Jun 20;2019:9271746. doi: 10.1155/2019/9271746. eCollection 2019. No abstract available. PMID: 31320907

Special Issue "IFN-Independent ISG Expression and its Role in Antiviral Cell-Intrinsic Innate Immunity". Thomas E, Saito T. Viruses. 2019 Oct 24;11(11). pii: E981. doi: 10.3390/v11110981.

Increasing uptake of evidence-based screening services though a community health worker-delivered multimodality program: study protocol for a randomized pragmatic trial. Carrasquillo O, Seay J, Jhaveri V, Long T, Kenya S, Thomas E, Sussman D, Trevil D, Koru-Sengul T, Kobetz E. Trials. 2020 Apr 29;21(1):368.

Patents: None at the time of reporting

3. **Grant #:** 7BC04 A Multilevel Intervention to Increase Colorectal Cancer Screening in Community Clinics

   Principal Investigator: Clement K. Gwede, RN, MPH, PhD, FAAN

   Organization: H. Lee Moffitt Cancer Center and Research Institute

   Grant Progress Report: Phase 1 (Preparatory) implemented important initial processes to support the conduct of Phase 2 RCT and was completed in previous reporting periods. This report centers on continuation activities for Phase 2 as summarized below:

   To test whether C-CARES Plus (education + FIT + personalized components) compared with C-CARES (education + FIT) improves long-term FIT screening adherence among 328 individuals, 50-75 years of age, who are not up to date with CRC screening. A total of 2669 age-appropriate patients were approached. Of these, 901 were not interested and 1768 patients were then evaluated for eligibility. Of these, 1408 were ineligible for a myriad reason with the primary reason (72%; 1019/1408) being already current with the CRC screening. This left 360 eligible patients. A total of 328 were successfully enrolled. Of the 32 not enrolled, six declined/no longer interested, two couldn't be reached, two no longer eligible, and 22 were not enrolled since the 328 targeted accruals was achieved on 11/30/2019. The study is closed to enrollment and follow up continues.

   Fecal immunochemical test (FIT) screening uptake (entire study) at six months (baseline) was 69% (225/328). A total of 55 abnormal FIT kit results have occurred which includes both baseline and 12-months abnormal (46 from baseline and nine for 12-month follow-up), and 17 participants have completed colonoscopy screening. Of the 38 uncompleted colonoscopies, 22 participants are pending and being tracked for colonoscopy completion either by the Moffitt team or navigated through the clinics' usual care practice. Another 16 patients are no longer being tracked due to the following: unable to reach after multiple contacts [n=3], refused colonoscopy [n=4] or per clinic procedure were considered resolved due to uncompleted colonoscopy despite multiple interactions with patients [n=9]).

   For C-CARES Plus arm, 3-6-month coaching intervention has been ongoing for those who did not return FIT at baseline for a cumulative total of 36 participants completed for the study duration. For 12-month coaching, a cumulative total of 30 participants completed the 12-month assessment interval. The 12-month follow-up and booster education interactions began, in April 2019. In total, 68 participants completed surveys and a total of 99 booster educations (1-page educational leaflet) has been mailed. For C-CARES arm, a total of 112 participants were mailed a generic CRC message via post-card per protocol, and next interactions or assessments will occur at final follow-up (27 months).

   As far as impact to Floridians, the produced educational DVD and photonovella booklets are currently being used as the basis of the intervention and access to screening is provided

via the FIT test for study participants and in the clinics overall per usual care practices. FIT screening rate of 69% at baseline matches the State's general population average and far exceeds the rates of approximately 40% seen in Florida's Federally Qualified Health Clinics.

Follow-on Funding: NIH; CARES-REACH (Colorectal Cancer Awareness, Research, Education and Screening Rural Expansion, Access and Capacity for Health); Clement K. Gwede, PhD, Cathy D. Meade, PhD; $3,145,317

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

4. **Grant #:** 7BC06 Discovery of Marine Natural Product Antagonists of Survivin as Novel Cancer Therapeutics

   Principal Investigator: Amy E. Wright, PhD

   Organization: Florida Atlantic University

   Grant Progress Report: Natural products or their derivatives represent over 48% of clinically approved cancer chemotherapeutics. The HBOI marine natural products chemical library represents a diverse source of genetically encoded small molecules that have actively co-evolved with cellular targets involved in both cell survival and death. The nodal protein survivin has been identified as an important target for intervention in several cancers including breast, colon and lung. Its expression correlates to poor prognosis and has a role in the aggressiveness of these diseases. It plays key roles as an anti-apoptotic protein; in mitosis; in drug resistance; in angiogenesis and in DNA repair response. Several approaches to inhibit survivin' s multiple functions have been explored but there remain very few small molecules that antagonize the activity of survivin. It is the goal of this project to discover natural products that reduce levels of survivin in colon and lung cancer cells and to further evaluate their cellular properties. Discovery of such compounds will advance this field both in our understanding of basic biology of survivin and in clinical practice. It is our hypothesis that assaying chemically diverse natural products in the HBOI library will provide new lead molecules for therapeutic intervention against colon, lung and breast cancers through reducing the level of survivin. Cell line (Aim1). At the end of Year two our team had completed the screening campaign; At the end of Year one our team had fully implemented the high content imaging (HCI) assays in both the DLD-1 colon adenocarcinoma cell line and the A549 lung carcinoma testing over 3000 materials in each of two HCI based assays to detect fractions that can reduce the levels of survivin in the A549 or DLD-1 cancer cell lines (Aim 2). All actives were retested to confirm activity. Six compounds from the HBOI Pure compound library reduced the levels of survivin in the HCI assays. One hundred-seventy fractions from the HBOI Enriched Fraction Library were identified that reduce the levels of survivin by >50% versus controls in either the DLD-1 or A549 HCI without showing significant cytotoxicity at 5 µg/ml. In Year three an additional 45 fractions that originally showed strong cytotoxicity against both the A549 and DLD-1 cell lines at the screening concentration of 5 µg/ml were tested at lower doses (5, 2.5, 1.25 and 0.625 µg/ml) resulting in identification of 26 fractions that reduce the levels of survivin >50% at non-cytotoxic doses. Bioassay- guided fractionation (Aim 3) has been on-going over years two and three and has led to the identification of 17 pure active compounds from twelve organisms in total 23 compounds have

been identified that reduce the levels of survivin by greater than 50% without showing significant cytotoxicity. Structure elucidation continues on three additional compounds. For three organisms, related inactive compounds have been identified providing early structure activity data. Progress towards Aim 4 includes completion of Western Blot analysis on the six active compounds identified from the HBOI pure compound library (n=3). Western Blot analysis has also been conducted on a subset of the most active fractions and purified natural products (n=1). IC50 determinations in the HCI assays have been completed for sixteen of the pure compounds. Initial work on the mechanism of action of the pure compounds has begun and will continue in the final grant period.

Follow-on Funding: HBO1 Foundation/IRL Grant Program; Discovery of survivin-targeting marine natural products from the Indian River Lagoon; Tandberg/McCarthy; $9250

Collaborations: None at the time of reporting

Journals: Guzman EA, Pitts, TP, McCarthy, PJ, Pomponi, SA, Diaz, CM, Reed, JK, Tandberg K., Wright AE, Screening and discovery of marine natural products that reduce the levels of survivin in the DLD-1 and A549 cancer cell lines Part I. Marine Drugs (covers all the compounds the team has discovered in the HBOI Pure Compound Library). This paper is 90% complete.

Tandberg K., Pitts, TP, Reed, JK, Guzman EA, Wright AE, Cholestenones from the Octocoral *Ellisella plexauroides* reduce levels of survivin in cancer cells. Winder PW, Barrett N, Pitts, TP, Diaz CM, Guzman EA, Wright AE 2,4-Imidazolidinediones from the marine sponge *Clathria* sp. reduce the levels of survivin in tumor cells.

Roberts, J, Pitts, TP, Diaz CM, Winder, PW, Guzman EA, Wright AE, Terpenes from the sponge *Phlyctaenopora halichondrioides* sp. that reduce the levels of survivin in cancer cells.

Guzman EA, Pitts, TP, McCarthy, PJ, Pomponi, SA, Diaz, CM, Reed, JK, Tandberg K., Wright AE, Screening and discovery of marine natural products that reduce the levels of survivin in the DLD-1 and A549 cancer cell lines Part II. Marine Drugs (covers the known compounds we have discovered through bioassay-guided fractionation).

Patents: None at the time of reporting

Bankhead-Coley Cancer Biomedical Research Program
Appendix E
Fiscal Year 2019-2020 Active Grants
Funding Fiscal Year 2015-2016

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
6BC04	University of Florida	David D. Tran, MD, PhD	$ 1,784,753.25	$ 1,403,290.34	$273,597.51	3/04/2016	3/31/2021	Yes	Yes	Yes
6BC06	University of Miami	Xiangxi Xu, PhD	$ 1,784,945.19	$ 1,412,267.94	$264,800.25	4/15/2016	3/31/2021	No	Yes	No
6BC09	University of Florida	Walter O'Dell, PhD	$ 1,445,736.61	$1,136,733.16	$221,627.25	3/19/2016	3/31/2021	No	Yes	No

1. **Grant #:** 6BC04 Novel Strategies to Target Disseminated Tumor Cells in Triple Negative Breast Cancer

   Principal Investigator: David D. Tran, MD, PhD

   Organization: University of Florida

   Grant Progress Report: The IL-6 Inhibitor Trial in TNBC: NCI funding started on 6/15/2020 and the protocol has received IND approval from the Food and Drug Administration (FDA) and Institutional Review Board (IRB) approval and officially open in early July. Subject recruitment and enrollment will commence in August 2020. The research staff will apply NETZEN and VEF (see below) to analyze patient samples obtained from this trial.

   NETZEN's web-based application: Research staff has now completed a beta version of the web-based working model of NETZEN. GeneRep, a key component of NETZEN, completed validation. nSCORE validation using >3400 gene profiles are progressing well with planned completion by the end of the summer 2020. Biological validation of nSCORE will be completed by year end.

   Development and Validation of a Virtual Experimentation Framework (VEF): The classical approach to determining and confirming cancer master regulators has focused largely on depleting and expressing individual genes and measure their impact on the survival and other malignant properties of cancer cells. Besides being time-consuming and resource-intensive, it is exceedingly difficult using this approach to resolve the extreme heterogeneity observed across tumors and patients to identify master regulators cooperating in unique manners in individual patients. Therefore, there is a great need for innovative computational algorithms that can perform virtual gene manipulation experiments to accurately and efficiently predict these patient-unique sets of master regulators. The research team has been working to create a novel Deep Probabilistic Neural Network (dpNN) based VEF. A beta version of the VEF was created to predict combinations of master regulators for aggressive cancers such as glioblastoma and breast cancer. Biological validation has resumed after COVID19-related disruption and is anticipated to be completed by early next year.

   Common master regulators (MR) of poor prognosis in 28 major human cancers: The research team has created reference networks for all 28 human cancers, which will be released for public access. The team has completed the initial analysis of the 28 reference networks and identified MR that predict poor prognosis. These MR are thus outstanding therapeutic targets. Importantly, human cancers can be grouped according to their MR of poor prognosis. Currently, the research team is working to identify common sub-pathways that transcend individual cancers that can be used for therapeutic development.

   Deep learning AI in EMR to predict health outcomes in human cancers, using breast cancer as a test case: The research team has formed a collaboration with the Breast Pathology group, the IT/Imaging group at UF, and the OneFlorida data trust to create a unique dataset of breast cancer pathological images and mammograms to integrate with a new AI algorithm that the team are developing to mine these large datasets. The research team will use >70K mammogram images and create a bank of high-resolution pathological slide images of at least 1000 patients with breast cancer to predict diagnostic criteria, disease course and treatment responses. This technology will have direct clinical applications if successful.

Follow-on Funding: NIH/NCI; Novel Methods of Chemo-sensitizing Low-proliferative Disseminated Tumor Cells in Triple Negative Breast Cancer; David Tran; $2,830,505

NIH/NCI; Targeting glioblastoma stem-like cells with custom-designed viral vectors; David Tran; $2,000,000 for Phase 2

AACR; Molecular mechanism of resistance to Tumor Treating Fields in glioblastoma; David Tran; $250,000

Sarepta; Artificial intelligence-directed approach in human tissue manufacturing for therapeutic targeting; David Tran; $1,056,398

Collaborations: Daohong Zhou, MD, College of Pharmacy, University of Florida.

Kian Huat Lim, MD, PhD, Molecular Oncology, Department of Medicine, Washington University in St Louis.

Zhijian Qian, PhD, College of Medicine, Department of Medicine, University of Florida Trey Ideker, PhD, Department of Medicine, UCSD, San Diego, CA.
Eric Vitriol, PhD, College of Medicine, Department of Anatomy and Cell Biology, University of Florida. Serge Zolotukhin, PhD, Department of Pediatrics, College of Medicine, University of Florida.

W. Gregory Sawyer, PhD, Department of Mechanical and Aerospace Engineering, College of Engineering, University of Florida.

Stephen Eikenberry, PhD, Department of Astronomy, College of Arts and Sciences, University of Florida.

Journals: Dan Jin, Nguyen Nguyen, and David D. Tran* (2019). Combining CDK4/6 inhibition with Cytotoxic Agents Does Not Enhance Cytotoxicity. *PLOS One*. October 10, 2019 *Senior/corresponding author.

Vinay K. Puduvalli, Jing Wu, Ying Yuan, Terri S. Armstrong, Elizabeth Vera, Jimin Wu, Jihong Xu, Pierre Giglio, Howard Colman, Tobias Walbert, Jeffrey Raizer, Morris Groves, David Tran, Fabio Iwamoto, Nicholas Avgeropoulos, Nina Paleologos, Karen Fink, David Peereboom, Marc Chamberlain, Ryan Merrell, Marta Penas Prado, W.K. Alfred Yung and Mark R. Gilbert (2019). A Bayesian Adaptive Randomized Phase II Multicenter Trial of Bevacizumab with or without Vorinostat in Adults with Recurrent Glioblastoma. *Neuro-Oncol*. Revision.

Patents: Tran, D.D. *"Master Regulators of Breast Cancer Metastasis."* March 2020, Serial No. 62/985,785.

Tran, D.D. and Le, S. "*Immunotherapy for Direct Reprogramming of Cancer Cells Into Immune Cells/Antigen Presenting Cells/Dendritic Cells*." December 2019: No. 62/952,725.

Tran, D.D. and Chen, D. *"Methods for Reducing Viability of Cancer Cells by Activation of the STING Pathway with TTFields."* November 2019: Serial No. 16/673,246.

Tran, D.D., Chen, D, and Le, S. "*Inhibiting Prostaglandin E Receptor 3 Resensitizes Resistant*

Cells to TTFields and Prevents Cells from Developing Resistance to TTFields." U.S. Provisional Application filed May 2019: Serial No. 62/849,535.

Zolotukhin, S and Tran, D.D. "*AAV capsid variants targeting human glioblastoma stem-like cells.*" Provisional Application filed August 2019: Serial No. 62/884,716
Exclusive licensing agreement to Lacerta Therapeutics, December 2019.

2. **Grant #:** 6BC06 Therapeutic Targeting of RAGE in Breast Cancer Progression and Metastasis

   Principal Investigator: Xiangxi Xu, PhD

   Organization: University of Miami

   Grant Progress Report: In the current funding period, regardless of the major restriction in place due to the COVID19 lockdown, our team has made major progress with testing our RAGE inhibitors in breast cancer cell models and in animal models of breast cancer. In cell assays, our team has further performed functional assays that show RAGE inhibitors impair metastatic function of breast cancer cells. Further, our team has made new discoveries with respect to the amount of drug required to impar metastasis with RAGE inhibitors in animal models and their impact on tumor cell growth and progression.

   Follow-on Funding: None at the time of reporting

   Collaborations: None at the time of reporting

   Journals: None at the time of reporting

   Patents: None at the time of reporting

3. **Grant #:** 6BC09 Early Markers of Subclinical Pulmonary Vascular Radiation Toxicity in Breast Cancer

   Principal Investigator: Walter O'Dell, PhD, and Julie Bradley, MD

   Organization: University of Florida

   Grant Progress Report: The goal of our project is to quantify early markers of lung radiation toxicity both to lung tissue and to pulmonary vasculature and relate these to blood markers of tissue inflammation, clinical signs of loss of respiratory function, and patients' quality of life. Our target population is women with breast cancer who are receiving either conventional X-ray based radiation or proton therapy to the chest wall and lymph nodes of the arm pit and under the sternum. Using tools developed in our lab to extract tissue and vessel response from chest CT scans, our team is able to quantify the severity of damage and to test the hypothesis that proton therapy leads to reduced severity both overall and as a function of radiation dose locally to different parts of the lung. To-date, 37 subjects have been enrolled and provided at least a baseline scan and marker panel. Our team has now completed analysis of 25 patients with a minimum of six- months follow-up. Enrollment was completed in February of 2020 to ensure a minimum of one-year follow- up by the project end data of March 2021. Scans, blood samples, clinical pulmonary function tests and quality of life surveys will be acquired out to two years

post-radiation treatment. New, more patient-specific models of tissue response have been formulated and applied to the currently collected data. The models will allow for more personalized assessment of a patient's risk and long-term quality of life based on the patients' total and regional dose, age, smoking history, X-ray versus proton therapy, and early blood markers of inflammation. A team of undergraduate and graduate students have been recruited to help with the data extraction and analysis. Several have received special funding from the University of Florida to pursue independent research projects around this topic. Our team has also developed and implemented to assess radiation toxicity to the heart in similar patients receiving X-ray versus proton therapy for breast cancer. Using more sophisticated and precise analysis methods than available currently in the clinic, our early results show, for the first time, a consistent decrease in global heart function in patients receiving conventional X-ray therapy, but a near-uniform improvement in heart function in proton therapy patients at matched time points post-treatment. Our team is now tailoring our tissue analysis tools for use in monitoring the long-term consequences of COVID-19 on lung and heart function.

Follow-on Funding: None at the time of reporting

Collaborations: Shruti Siva Kumar successfully defended a PhD thesis in July 2020, from the Department of Biomedical Engineering at UF. Dr. Kumar's efforts were covered by this award and Dr. Kumar' written thesis contains a plethora of analysis results that the team will be sifting through over the next few months for public presentation and publications.

Brandon Terracino is a Medical Physics PhD student at UF and Mr. Terracino's thesis work is on in lung tissue radiation damage with high-dose proton therapy, which is related to this award.

Aren Saini is a biology pre-med undergraduate student who is working on application of the vessel extraction and sizing methods to pediatric patients. From 2018-2019 Aren was funded through a UF University Scholars Program Award for this work.

Shoba Abraham is a UF undergraduate pre-med student who received a University Emerging Scholars Program award to conduct BHC-related research in our lab for 2019. For the 2020-2021 academic year Ms. Abraham was awarded a UF Health Cancer Center University Scholars Program Award to extend this work.

Siri Ravuri is a UF undergraduate pre-med student who is analyzing pulmonary vascular changes around developing tumors. In 2018 Siri was awarded a UF Cancer Center University Scholars Program Award for this work and Ms. Ravuri was recently awarded a 2020-2021 UF University Scholars Program Award to extend this work.

Enrico Bautista is a UF undergraduate in physics who was recently awarded a 2020-2021 UF University Scholars Program Award to study the effects of breathing on dose deposition in the lung during proton therapy.

Samual Martucci is a recent graduate from UF in biomedical engineering and will be starting in the BME graduate, master-degree program this fall. Mr. Martucci has been pursuing research to characterize the effect of different imaging conditions on the accuracy of pulmonary vessel sizing.

Our recent abstract submissions include efforts of new early-career radiation oncology clinicians at the UF, Drs. Natalie Lockney, Ray Mailhot and Michael Rutenberg. It includes also new team members medical physicist Xiaoying Liang.

Journals: Begosh-Mayne D, Toffel S, Siva Kumar S, Okunieff P, O'Dell W. A Comparison of Dose-Response Characteristics of Four NTCP Models: Using a Novel CT-based Radiomic Method to Quantify Radiation- Induced Lung Density Changes. Published in *Nature: Scientific Reports,* May 25, 2020. Available on-line June 29, 2020 doi: 10.1038/s41598-020-67499-0

Saini A, Siva Kumar S, O'Dell W. Measuring Lung Vessel Tree Growth During Development in Pediatric Patients. *University of Florida Journal of Undergraduate Research* 2020;21.2 doi: 10.32473/ufjur.v21i2.108563

Siva Kumar S and O'Dell W. A Systematic Review of Normal Tissue complication probability models for predicting radiation-induced cardiac and lung injury in breast cancer patients Submitted to *Radiotherapy and Oncology*, June 24, 2020, currently under revision.

Patents: None at the time of reporting

Bankhead-Coley Cancer Biomedical Research Program
Appendix F
Fiscal Year 2019-2020 Active Grants
Funding Fiscal Year 2014-2015

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
5BC07	H. Lee Moffitt Cancer Center	Eric Haura, MD	$1,686,887	$ 1,428,263.25	$95,217.55	5/25/2015	5/15/2021	No	No	Yes

1. **Grant #:** 5BC07 Signaling-Associated protein complexes for the molecular annotation of therapeutic vulnerabilities, resistance-associated signaling and tumor heterogeneity in lung cancer

   Principal Investigator: Eric B. Haura, MD

   Organization: Moffitt Cancer Center

   Grant Progress Report: The overarching goal of this project is to utilize signaling-associated complex assays to interrogate aberrant oncogenic signaling in lung cancer. Understanding how proteins interact with each other in functional complexes can help drive development of biomarkers and diagnostic assays to stratify patients. AXL is an attractive drug target because of its role in resistance to targeted therapy in various cancers. However, there is limited understanding of AXL's interacting partners. In this study, our team used the BioID system, which utilizes a proximity-dependent labeling strategy, to map out the AXL interactome in a lung cancer cell line model. The AXL interacting proteins could be classified majorly into tight junction organization (consisting of OCLN, TJP1, NUMB, LLGL1, AFDN, ITGB1, ITGA2, CTTN, SCRIB), cell migration (consisting of ERBB2IP/ERBIN, CD44, EPHA2, EPB41L5, AFDN, ITGB1, ITGA2, CTTN, SCRIB) and protein localization to cell periphery (consisting of EPB41, EPB41L2, ITGB1, EGFR, DLG1) clusters. Our team identified tight junction organization and protein localization to the cell periphery as the top enriched functional modules from KEGG and GO analyses, thereby suggesting that AXL has a major role to play in regulating cell adhesion and migration in these cells. Our team is under the process of submitting a manuscript describing the data from this study. This approach can be applied in future to identify functional signaling complexes for other relevant drug targets which in turn can guide development of assays such as proximity ligation assays for in situ detection of active signaling foci. Many covalent inhibitors are in clinical trials for KRASG12C mutants and early clinical data indicate some positive results, yet evidence of rebound is also reported. The research funding from Bankhead-Coley was utilized to elucidate short-term signaling adaptations to KRASG12C specific inhibitor in lung cancer. Initial findings, presented in AACR 2020, are encouraging where our team reports that irrespective of having common tumor driving G12C mutation in KRAS, lung cancer cells respond differentially to KRASG12C inhibition. Our team was able to differentiate KRASG12C lung cancer models in to epithelial-mesenchymal subtype, where epithelial subtype use ERBB signaling as therapy resistance and benefit from dual pan-ERBB/G12Ci combination. In contrast, Mesenchymal subtype likely to use Fibroblast Growth Factor Receptor 1 (FGFR1) signaling as therapy adaptation and would benefit from dual FGFR/G12Ci. Similar phenotypic differences were also observed in KRASG12C lung tumors in patients when our team analyzed TCC data set on lung adenocarcinoma. The findings of this study are currently being evaluated in cell line- and patient-derived xenograft models as a part of research collaboration with Janssen Pharmaceuticals. Further, research funding from Bankhead-Coley funding and NCI-supported UH2/UH3 will enable to characterize signaling complexes for ERBB and FGFR1. Assessing levels of these signaling complexes in KRASG12C lung cancer patient tissues might help in identifying personalized combination strategies. Research staff has made significant progress during this cycle of funding and in this final year of the grant our team is under process to submit manuscript and also look forward to apply our findings in clinical settings to guide biomarker development strategies to predict clinically effective combinations in diverse group of KRASG12C mutant lung cancer.

Follow-on Funding: NCI; Validation of EGFR Protein Complexes as Molecular Diagnostics; Eric B. Haura MD; $993,537

Collaborations: None at the time of funding

Journals: None at the time of funding

Patents: None at the time of funding

Bankhead-Coley Cancer Biomedical Research Program
Appendix G
Fiscal Year 2019-2020 Completed Grants
Funding Fiscal Year 2018-2019

Grant #	Organization	Principal Investigator	Award Amo	Life to Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
9BC02	Florida Atlantic University	Michael Lu, PhD	$58,162	$33,927.00	$24,235.00	5/30/2019	12/15/2019	No	No	No
9BC05	H. Lee Moffitt Cancer Center	Katarzyna Rejniak, PhD	$100,000	$93,828.89	$6,171.11	3/22/2019	9/30/2019	No	No	No
9BC06	H. Lee Moffitt Cancer Center	Paulo Rodriguez, PhD	$100,000	$99,999.99	$0.01	3/22/2019	9/30/2019	No	No	No
9BC10	Mayo Clinic	Peter Storz, PhD	$85,409	$85,409.00	$0.00	3/26/2019	9/30/2019	No	No	Yes
9BC11	University of Miami	Izidore Lossos, MD	$100,000	$50,000.00	50,000	6/4/2019	10/31/2019	No	Yes	Yes
9BC15	University of South Florida	Minjung Kim, PhD	$100,000	$97,492.35	$2,507.65	5/29/2019	10/31/2019	No	No	Yes

1. **Grant #:** 9BC02 PAK6 in Advanced Prostate Cancer

   Principal Investigator: Michael L. Lu, PhD

   Organization: Florida Atlantic University

   Grant Progress Report: During the project period, the research team has finished building and characterizing the model cell line system that allows us to specifically probe the effects of p21-activated kinase 6 (PAK6) expression on regulating centrosome biogenesis. The model consists ectopically expressing PAK6 kinase Wild Type (WT), kinase dead and constitutive active mutants in HEK293 cells for determining the PAK6 kinase activity-regulated biological effects. Additional new discovery of PAK6 involvement in centrosome regulated ciliogensis was realized. The team validated effects of PAK6 in ciliogenesis and demonstrating its causal relationship. Our team has generated recombinant adeno-associated viral vectors to further our inquiry into the clinically relevant cell culture models to determine the role of dysregulated PAK6 activation in association with prostate cancer progression. Our team hypothesize that PAK6 functions as a negative regulator in centrosome amplification and microtubule dynamics. PAK6 overexpression-induced high-aneuploidy is a result of centrosome abnormality due to deregulated PAK6 activities. Aim1: Determine the functional role of PAK6 in maintaining centrosome homeostasis Aim 1a. Determine whether PAK6 signaling in a linear or parallel manner to the Mitotic/PLK-kinase-axis. This aim is designed to identify the specific PAK6 targets in the centrosome homeostasis pathway. Since centrosome duplication occurs in synchrony with the cell division cycle at S and G2 phases, the team will examine and map the potential crosstalk between PAK6 and Mitotic/PLK-signal axis using a centrosome amplification rescue assay. Aim 1b. Determine whether PAK6 overexpression induces premature or delayed centriole dis- engagement. This aim is to determine if PAK6 overexpression-induced high ploidy is a result of premature centriole disengagement. Aim 2: To identify and characterize molecular targets downstream of PAK6 pertinent to regulation of microtubule dynamics. A proteomic approach employing tandem affinity tagged PAK6 and tandem mass spectrometry will be used to identify PAK6 downstream candidate targets.

   Follow-on Funding: None at the time of reporting

   Collaborations: None at the time of reporting

   Journals: None at the time of reporting

   Patents: None at the time of reporting

2. **Grant #:** 9BC05 Metabolic Reprograming to Improve lmmunotherapy in Melanomas

   Principal Investigator: Katarzyna A. Rejniak, PhD

   Organization: H. Lee Moffitt Cancer Center and Research Institute

   Grant Progress Report: Our team investigated melanomas, which are aggressive tumors resistant to radiation and chemotherapy. lmmunotherapies to improve anti-tumor T-cell activity have led to durable responses in melanoma patients where conventional therapies have failed. However, the response rates remain low. Our research team hypothesized that specific metabolic conditions (hypoxia and acidity) in the melanoma microenvironment play an

immunosuppressive role, but carefully planned manipulation of the tumor microenvironment can result in improved melanoma response to immunotherapy. Our methods included computational simulations informed by experimental data to direct experiments with defined T-cells in a murine model of melanoma. During the six-months funding period of this Bridge grant, the team pursued research in both proposed aims in parallel. Our team has characterized changes in the properties of T-cells when exposed to various levels of acidic and hypoxic conditions in vitro, and how theses complex microenvironments influence production of IFN-gamma by the exposed T-cells. Our team used the in-silico model calibrated with the morphology and metabolism of in vivo B16 melanoma and predicted spatial distribution of IFN-gamma in digitized tumor histology. Our team has also demonstrated improved tumor response to combination therapy that includes hypoxia-activated pro-drugs and adoptive T-cell transfer. This Bankhead-Coley Bridge award allowed for generation of new preliminary data making applications for federal funding more competitive. This Bankhead-Coley Bridge grant allowed our team to demonstrate as a proof-of-principle that interventions guided by a mathematical model calibrated to specific experimental data lead to improved therapeutic outcomes. Our further studies will focus on optimizing schedules for combinations of multiple therapeutic options: adoptive T-cell transfer, checkpoint blockades, hypoxia-activated pro-drugs and bicarbonate buffers using our integrated approach. Our team will seek federal funding for the projects that stemmed from this award.

Follow-on Funding: None at the time of reporting

Collaborations: Vanderbilt University, Nashville TN, 1 undergraduate summer internship: Mr. Tamas Kis, Vanderbilt University, 05/21-08/01 2019, participated in the Moffitt Summer Program for the Advancement of Research Knowledge (SPARK) and conducted research in Dr. Rejniak lab. Mr. Kis was involved in the development of a mathematical model of T cell infiltration and IFN-gamma generation in a heterogeneous microenvironment of melanoma.

Journals:
Hybrid modeling frameworks of tumor development and treatment. Chamseddine, I., Rejniak, K.A., WIRES Systems Biology and Medicine. July 2019.

Patents: None at the time of reporting

3. **Grant#:** 9BC06 Functional reprogramming of tumor-MDSC through antibody-based therapies targeting Notch

Principal Investigator: Paulo, C, Rodriguez, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Grant Progress Report: Throughout this bridge grant, our team aimed to identify the signals by which the expression of the Notch ligands, Jagged1-2, in tumor-bearing hosts functionally drives Myeloid Derived Suppressor Cell (MDSC) functionality. Our overall hypothesis was that the expression of Jagged1-2 in cancer cells plays a central role in the suppression of protective T-cell immunity in tumors. Our team proposed the following Aims: Determine the therapeutic effect of humanized anti-Jagged 1-2 blocking antibodies in tumor-bearing mice; 2) Elucidate the mechanistic interaction between the expression of Jagged1-2 in cancer cells and MDSC immunosuppression in tumors; and 3) Investigate the role of the Sting-associated Type I interferon production as the driver signal mediating the immunogenic effects induced by anti-

Jagged1-2 therapy in tumor-bearing hosts. The immunosuppressive microenvironment developed in epithelial tumors remains a major limitation for the potential benefit of promising cancer therapies, including immunotherapy. Among the multiple populations impairing immune surveillance in the tumor beds, Myeloid-derived suppressor cells (MDSC) have emerged as major regulators of T, NK, and B-cell anti- tumor potential. Although several approaches have been proposed to therapeutically blunt the regulatory activity of MDSC, there are no effective treatments to clinically block MDSC in cancer patients. In this 6-month bridge grant, our team aimed to evaluate whether the expression of the Notch ligands, Jagged 1 and 2, in the tumor microenvironment plays a role in the polarization of MDSC into highly immunosuppressive populations. Our research team proposed the following Aims: 1) Determine the therapeutic effect of humanized anti-Jagged1-2 blocking antibodies in tumor-bearing mice; 2) Elucidate the mechanistic interaction between the expression of Jagged1-2 in cancer cells and MDSC immunosuppression in tumors; and 3) Investigate the role of the STING-associated Type I interferon production as the driver signal mediating the immunogenic effects induced by anti-Jagged1-2 therapy in tumor-bearing hosts.

The major goal of this application was to complete the experiments that could allow our team to address the favorable comments of the reviewers for the R01 application CA233512 and that will enable the submission of a revised R01 proposal. The funds provided by the bridge program of the BHC-CR have been primary for the completion of this goal. Because the revised R01 application has been submitted and funded, our team will now focus on completing the proposed research and extend the program into further applications and potential clinical assays. Indeed, our team recently submitted a new application testing the effects of the activation of Notch signaling in the therapeutic activity of CAR T cells in ovarian carcinoma (R01-CA248079).

Follow-on Funding: NIH/NCI: Functional reprogramming of tumor-MDSC through antibody-based therapies targeting NOTCH Ligands: Paulo C. Rodriguez, PhD: $1,143,750.

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

4. **Grant #:** 9BC10 Role of ICAM1 in Development and Progression of Pancreatic Cancer

   Principal Investigator: Peter Storz, PhD

   Organization: Mayo Clinic

   Grant Progress Report: With the support of the Bankhead-Coley bridge grant, our team successfully obtained federal follow-up funding (R01, see below). This will allow the team to continue and complete our project and to hire additional personnel to work on this project. With the help of the bridge grant our team has generated tools needed and obtained data that confirm our hypotheses. There are no publications or presentations to report during the time span of the six-month funding period.

   This Bankhead-Coley grant is a bridge funding grant for our R01 proposal that has four Specific Aims. These Specific Aims are designed to test the hypothesis that KRas-driven expression of

ICAM-1 is a regulator of macrophage populations and its targeting can have major effects on development and progression of pancreatic cancer. With the three Specific Aims from the bridge funding the team started working on Specific Aims 1. 3 and 4 of the initial R01 proposal, because these Aims were critical for a successful resubmission of the R01 grant proposal. With the support of the Bankhead-Coley bridge grant, our team successfully obtained R01 funding. Our team is now in a position to finish our project to a stage where our team eventually will evaluate if neutralizing of the molecule ICAM1 can be an option for combination therapy to extend the survival of pancreatic cancer patients.

Follow-on Funding: NIH/NCI: Role of ICAM1 in development and progression of pancreatic cancer: Peter Storz PhD; $1,789,970.

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

5. **Grant #:** 9BC11 Identify the Mechanisms of LMO2-Mediated Inhibition of Homologous Recombination and Establish PARP-Targeted Synthetic Lethality as a New Therapy for DLBCL

   Principal Investigator: Izidore Lossos, MD

   Organization: University of Miami

   Grant Progress Report: Compromised Homologous Recombination (HR) can contribute to genomic instability and increase the mutational burden. To determine whether primary primary LIM Domain Only-2 (LMO2)HIGH Diffuse Large B Cell Lymphoma (DLBCL) tumors show an increase in somatic mutations when compared to $LMO2_{LOW}$ tumors, our team searched publicly available DLBCL datasets with information on LMO2 expression and somatic mutations in the same tumors (Chapuy et al., 2018). These analyses revealed a positive correlation between the number of somatic mutations and LMO2 mRNA expression in primary untreated DLBCL. Similarly, $LMO2_{HIGH}$ DLBCL tumors (defined as second and third tertial based on the spectrum of LMO2 mRNA expression) showed a statistically significant increase in the number of somatic mutations in $LMO2_{HIGH}$ DLBCL tumors. Further, in an independent cohort of DLBCL patients, our team also observed a statistically significant increase in the number of mutations per sequenced coding megabase (identified by FoundationOne Heme test) in $LMO2_{HIGH}$ DLBCL (using a previously defined 30% threshold via IHC for LMO2 protein expression). These observations suggest that LMO2 by inhibiting HR may lead to increased genomic instability not only in cell lines but also in primary DLBCL tumors.

   Follow-on Funding: NCI; Identify the Mechanisms of LMO2-Mediated inhibition of Homologous Recombination and Establish PARP-Targeted Synthetic Lethality as a New Therapy for DLBCL; Izidore Lossos, Raminor Verdun; $1,797,380

   Collaborations: None at the time of reporting

   Journals: MO2 Confers Synthetic Lethality to PARP Inhibition in DLBCL. SalmaParvin[19]ArielRamirez abrada[1289] ShlomzionAumann[12] XiaoQingLu[12] NataliaWeich[12]

GabrielSantiago[1] Elena M.Cortizas[1] EdenSharabi[1] YuZhang[1] IsidroSanchez-Garcia[3] Andrew J.Gentles[4] EvanRoberts[2] DanielBilbao-Cortes[2] FranciscoVega[25] Jennifer R.Chapman[25] Ramiro E.Verdun[12610] Izidore S.Lossos[1271011] https://doi.org/10.1016/j.ccell.2019.07.007

Patents: None at the time of reporting

6. **Grant #**: 9BC15 Testing the Value of PTPN11 as a Novel Therapeutic Target in BRAF Wild-Type Melanomas

   Principal Investigator: Minjung Kim, PhD

   Organization: University of South Florida

   Grant Progress Report: To develop strategies targeting *BRAF* wild-type melanoma by PTPN11 inhibition in this study, our team proposed to establish the importance of GSK3/13-catenin/cyclin D1 pathway in predicting the response and the resistance to PTPN11 inhibition (aim 1), to develop co-targeting strategies (ex. MEK, 13-catenin, or cyclin D1/CDK4 with PTPN11 to elicit better and more durable response (Aim 2), and to assess the tumor intrinsic and extrinsic mechanisms underlying the response to PTPN11 inhibition (aim 3). Under the support of Bankhead Coley Cancer Research program, our research team tested the effect of wild type or mutant (Y216E, Y216F, Y216, KSSR) forms of GSK313 on soft agar colony growth (Aim 1) and observed that phosphorylation defective (VF, YA) or kinase-dead (KSSR) GSK313 mutant drives colony growth, supporting the importance of kinase a activity and Y216 phosphorylation of GSK313 regulated by PTPN11. Expression of a 13- catenin stabilized mutant (S33A/S37A/T41A/S45A) in 5037 cells suppressed cell death and cyclin 01 down-regulation by SHP099. These data combined support the importance of the GSK3/13-catenin/cyclin 01 and RAS/RAF/MAPK pathways downstream of PTPN11 (Aim 1). In addition, our team tested the response to SHP099 (PTPN11 inhibitor) of several human melanoma cell lines. SBCL2 *(NRASQ61)* responded to SHP099, associated with downregulation of ERK, 13-catenin, cyclin D1 and upregulation of pY-GSK3, which was reversed by CHIR- 99021 (GSK3 inhibitor and was further enhanced by combined treatment with a MEK inhibitor, MEK162 (binimetinib) (Aim 2). Moreover, the team observed that combination of SHP099 with anti-PD1drastically suppressed the tumor growth on the lung without significant weight loss/side effects while either SHP099 or anti-PD1 alone also has mild growth inhibitory effects compared to control (Aim 2 and 3). The immune cell profiles showed increased cytotoxic CD+ T-cells and NK cells in lungs of mice treated with combination of anti-PD1 and SHP099. Depletion of NK cells a-NK1.1) markedly reduced the therapeutic effect of SHP099 and SHP099/a-PD1 combination, while depletion of CD4+ or CDS+ cells failed to affect tumor growth of SHP099 treated mice This suggests the important roles of NK cells in response to SHP099 and SHP099/ a-PD1 combination. Our team has treated melanomas developed in PTPN11 E76K mouse models with PTPN11 pharmacological (SHP099, NSC- S7S77) or genetic (doxcycline off) inhibition. These tumors are being analyzed for their impact on pERK, GSK3/cyclin D1, and immune profiles (Aim 3). Based on strong anti-tumor effect of SHP099 and anti-PD1 combination in 816F10 model, our focus has been shifted toward combine treatment of PTPN11 inhibition and immunotherapy. The team will confirm this treatment strategy in 5037 NRAS- riven tumors in syngeneic FVB/N mice, focusing on the long-term survival benefit. The team will assess molecular mechanisms and signaling axis of PTPN11 inhibitors in NK and T cells by. Specifically, the team will analyze effects of PTPN11 inhibitors in immune cells (CD4+ or CDS+ T-cells, NK- cells) on their proliferation/differentiation/effector function (cytotoxicity, secretion of cytokine/chemokine) and on signaling effectors (MAPK, JAK/S AT, ZAP70, LCK, GSK3/13 catenin/cyclinD1/3, CD2S,

PD1, CTLA4). This study will be reported in peer-reviewed journal later this year/early next year. Recently, our team obtained RMC-4550, a better and more effective PTPN11 inhibitor than SHP099, developed by Revolution Medicines, Inc. in collaboration. Our team is expanding the study by employing g additional PTPN11 inhibitors.

Follow-on Funding: Revolution Medicines, Inc; Preclinical studies of SOS1 inhibitor in SHP2-driven murine melanoma cell lines; Minjung Kim; $149,000

Collaborations: Our team established collaboration with Revolution Medicines, INC., a biotech company located in Redwood City, CA. This company has generated PTPN11 inhibitor, RMC-4550 and RMC-4630, which is in clinical trials. This company provided these PTPN11 (8HP2) inhibitors and 8081 inhibitors (RMC-0331) to test their impact on PTPN11-driven tumor cell lines along with funding.

Journals: None at the time of reporting

Patents: None at the time of reporting

Bankhead-Coley Cancer Biomedical Research Program
Appendix H
Fiscal Year 2019-2020 Completed Grants
Funding Fiscal Year 2017-2018

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
8BC02	H. Lee Moffitt Cancer Center	Brian Ruffell, PhD	$815,289	$397,350.38	$417,938.62	4/20/2018	3/31/2021*	No	No	No

***Terminated 9/15/2019**

1. **Grant #:** 8BC02 Regulation of Dendritic Cell Function and Tumor Immunity by TIM-3

   Principal Investigator: Brian Ruffell, PhD

   Organization: H. Lee Moffitt Cancer Center and Research Institute

   Grant Progress Report: We have made major strides in confirming the two-underlying hypothesis of our grant, namely that T-cell immunoglobulin mucin-3 (TIM-3) negatively regulates the uptake of extracellular DNA by dendritic cells, and that TIM-3 blockade functions therapeutically by improve the colocalization of dendritic cells and T cells within tumors. Regarding the first hypothesis, our data now shows that TIM-3 blockade leads to the uptake of DNA by bone marrow-derived dendritic cells and splenic dendritic cells. We have also found that the absence of STING (STtimulatory of InterferoN Genes) prevents TIM-3 blockade from inducing expression of CXCL9 in vitro and abrogates response to combinatorial therapy with TIM-3 blockade paclitaxel. We anticipate completing Aim 1 within the next 12-month period and publishing our results in a high impact journal. Regarding our second hypothesis, we established a system to map the localization of each dendritic cell and T cell within a whole tumor section and analyze the distance between cells along with the size of T cell clusters around each dendritic cell. Both parameters were altered by TIM-3 blockade and paclitaxel chemotherapy in a pilot study, and we are now working on validating these results and expanding our studies to determine the role of chemokine expression on this phenotype. No significant changes in personnel, programs, or shared resources have occurred during this funding period that would impact our research. We have mostly completed experiments described in Aim 1 of the proposal and a manuscript describing these studies is currently in revision at a high impact journal. In the next 12 months we plan to complete key experiments described in Aim 2 of the proposal, focusing on the role of intratumoral Dendritic Cells (DCs) and the importance of chemokine expression in DC-T cell colocalization within tumors. We will also determine if TIM-3 blockade (and by extension activation of the STING pathway) leads to alterations in DC migration, antigen presentation or cytokine production, based upon preliminary data suggesting that additional pathways are activated by TIM-3 blockade. In the next 12-month period we plan to finalize our experimental results and submit our 2nd article for publication describing the importance of DC-T cell clustering in the anti-tumor response following TIM-3 blockade. We hope to extend these findings to explore the relationship between DC-T cell colocalization in response to other immune checkpoint blockade therapies to determine if this is broadly applicable.

   Follow-on Funding: NIH/NCI; Determining the role of intratumoral dendritic cells in anti-tumor immunity; Alycia Gardner; $105,885

   NIH/NCI; Determining the role of dendritic cells and spatial localization in anti-tumor immunity; Alycia Gardner; $71,052

   NIH/NCI; Regulation of dendritic cell function and tumor immunity by TIM-3; Brian Ruffell; $1,967,250

   CDMRP/DOD; Breast Cancer Immunotherapy with anti-TIM-3; Brian Ruffell; $774,000.

Collaborations: None at the time of reporting

Journals: Extracellular DNA update and STING activation in dendritic cells is suppressed by TIM-3. Alvaro de Mingo Pulido, Bruna Batista-Bittencourt, Reymi Pena, Jimena Trillo- Tinoco, Eslam Mohamed, Alycia Gardner, Kay Hiinggi, Asmaa El-Kenawi, Tsuneyasu, Kaisho, Matthew F. Krummel, Johanna Kaufmann, Kristen McEachern, Hatem Soliman, Vincent C. Luca, Xiaoqing Yu, Paulo C. Rodriguez, Brian Ruffell. Immunity 2019.

Patents: None at the time of reporting

Bankhead-Coley Cancer Biomedical Research Program
Appendix I
Fiscal Year 2019-2020 Completed Grants
Funding Fiscal Year 2016-2017

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
7BC01	University of Miami	Anthony Capobianco, PhD	$ 1,471,318	$ 1,389,577.50	$ 81,740.50	3/17/2017	2/29/2020	Yes	No	No
7BC05	H. Lee Moffitt	Keiran Smalley, PhD	$ 1,468,200	$ 1,223,500.00	$244,700.00	4/12/2017	2/29/2020	No	Pending*	No
7BC08	H. Lee Moffitt Cancer	Shari Pilon-Thomas, PhD	$ 976,620	$ 922,352.00	$ 54,268.00	3/08/2017	2/29/2020	No	No	No

*Articles have been accepted but have not been published yet.

1. **Grant #:** 7BC01 Development of Small Molecule Inhibitors of NACK as Novel Cancer Therapeutic Agents Targeting the Notch Pathway

   Principal Investigator: Anthony J. Capobianco, PhD

   Organization: University of Miami Miller School of Medicine

   Grant Progress Report: Our team optimized our virtual model via extended molecular dynamics (MD) simulations using the recently deposited apo NACKcrystal structure (PDB ID: 5VE6). Using this model, a library of 20 compounds was designed and synthesized. All compounds were screened in the colony formation assay to measure cell viability, and in an LCK Kinase Glo assay for selectivity. Analogue UM-74 displayed enhanced activity with a cell-based ICso of 608 nm. The information obtained from screening this second library has enabled the team to further optimize our virtual model and informed the design of a third library of inhibitors. This third library has been synthesized Our team developed the first label-free NACK binding assay using a Differential Scanning Fluorimetery (DSF) assay where the team has prioritized compounds based on relative KD (equilibrium dissociation constant). Preliminary studies suggest that analogue UM-159 binds to NACK with an enhanced affinity by two orders of magnitude in comparison to hit compound 2271-0326.Our team is currently screening these prioritized compounds in colony formation assays to determine activity. Moreover, our team has additional data to suggest that Z271-0326 and analogues bind in a type II conformation. This is illustrated by our DSF assay results which display a left destabilizing shift. Our team also identified that NACK binds staurosporine and AMP-PNP which results in a stabilizing right shift typical of type I kinase inhibitors. Our team screened controls and optimized compounds in a DSF assay with a catalytic lysine mutant (K1024A), and observed no shifts for controls or analogues, suggesting binding is occurring in the ATP pocket. For our cell-based assay, our team included MDA MB 231 Triple Negative Breast (TNBC). The assays have been tested in colony formation and cell titer glow assay. PC3 prostatic cancer cell lines were recently included in our screening as iNACK nonresponsive cell lines. Our team will continue to screen the third inhibitor library in Notch rich OE33 and MDA-MD-231, as responsive cell lines, and PC3, prostatic cancer, as un-responsive cells, to determine activity of the optimized inhibitors and select against toxicity. Additional compounds will be synthesized to provide further proof to our binding hypothesis which includes synthesis of an indolizine scaffold, and a methyl protected acetamide. Our team will continue to optimize our NACK DSF assay and optimize parameters such that our team may obtain KDs with confidence. Additional studies with NACK point mutants will also be performed in the DSF assay in order to provide additional insight into our binding hypothesis. These mutants have been constructed and are single mutations of residues predicted by our virtual model to be necessary for binding. Our lead preclinical compound will be identified by selecting the analogue with best NACK KD, best activity in cells with no apparent off-target cytotoxicity. These inhibitors will be submitted for ADMET to Dr. Michael Cameron at The Scripps Research Institute Florida. Analogues with favorable ADMET will be administered to OE Xenograft mice to evaluate in-vivo activity. Additionally, our team will continue to optimize our virtual model. Our team has started a new collaboration with Prof. Dr. Stefan Knapp at Goethe University where the team's protein crystallization group is actively screening NACK and analogues for crystal formation. The team hopes to obtain a NACK-analogue X-ray structure to incorporate into our virtual model. If the team cannot obtain a crystal, the team will continue with our previously described plan to optimize the existing NACK virtual model through template threading.

   Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

2. **Grant#:** 7BC05 Defining and Targeting Epigenetic Deregulation in Uveal Melanoma

   Principal Investigator: Keiran S.M. Smalley, PhD

   Organization: Moffitt Cancer Center

   Grant Progress Report: Uveal melanoma is the deadliest form of eye cancer. In 50% of cases it metastasizes, overwhelmingly to the liver. Once in the liver, uveal melanoma is almost completely refractory to treatment. The overarching hypothesis of this grant is that the genes responsible for driving uveal melanoma convey a dependency on a class of epigenetic enzymes called the Histone DeACetylases (HDACs) which might represent a therapeutic vulnerability. The team further hypothesized that HDACs are required for uveal melanomas to adapt to a potentially promising therapy for uveal melanoma (MEK inhibitors) and that co-targeting of HDACs and MEK will lead to durable therapeutic responses.

   In this grant the team used state-of-the-art proteomic and gene expression analysis techniques to determine how uveal melanoma cells were able to overcome the effects of MEK inhibitor therapy. The team identified several new mechanisms of therapeutic escape including growth factors directly secreted from the cancer cells, and increased survival signaling that resulted from the cancer cells re-arranging their own structure (cytoskeletal re-arrangement). The team determined that these effects were dependent upon HDACs, and that use of HDAC inhibitors (which are in clinical use) could prevent therapeutic escape. Out team then demonstrated that the combination of a MEK and HDAC inhibitor was effective at shrinking established uveal melanomas in mice. Of note, our team developed a new mouse model of uveal melanoma growing in the livers of mice and showed that the MEK-HDAC inhibitor drug combination was effective against these most intractable of tumors.

   In parallel to these efforts the team also performed unbiased genetic Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) screens to identify other targets that could synergize with MEK inhibitors. The purpose of this experiment was to determine if the team would identify targets that were more effective than the HDACs. These screens identified other potential genetic regulators that the staff then validated alone and in combination with MEK inhibitors in our uveal melanoma mouse models. Among the drug combinations the team tested, the team found that decitabine (another FDA-approved drug) synergized effectively in mouse models.

   In addition to tumor intrinsic mechanisms of drug resistance, there is also evidence that normal host cells can also contribute to drug resistance in cancer cells. To evaluate this, the team used a new technology called single cell RNA-Seq which allows the gene expression profiles of individual cells in tumors to be analyzed. Using this approach, the team identified several new pathways and genetic regulators in both human uveal melanoma specimens and mouse tumors that has formed the basis for a new collaborative grant submitted by this group to the National Institutes of Health.

Through the course of this grant our staff have identified several promising new drug combinations. Working with our clinical colleagues at Moffitt Cancer Center and the University of Miami our team has planned a new phase II clinical trial of the MEK inhibitor Binemetinib in combination with the HDAC inhibitor Belinostat that should be opening shortly. This trial will lay the groundwork for a new family of therapies that offers hope for Floridians with uveal melanoma.

Follow-on Funding: None at the time of reporting

Collaborations: This project is a collaboration between the H. Lee Moffitt Cancer Center, The University of Florida and the University of Miami. The labs of the three principal investigators have monthly one-hour virtual lab meetings using the Zoom teleconferencing system to discuss new data and to share experience relevant to the project. Since initiation of the project the groups have met 2-3 times per year, including a one-day retreat in Tampa in Nov 2017, the FACCA meeting in Tampa in April 2018 and the Miami Epigenetics Meeting in November 2018. The group met in person at the annual FACCA retreat in Miami in June of 2019 and at the SMR annual meeting in Salt Lake City, UT in November 2019. Dr. Smalley traveled to UF in December 2019 to meet with Dr. Licht and the team. On January 25, 2020, all three groups are meeting at the UF meeting facility in Orlando for a one-day scientific retreat.

Journals: HDAC inhibition enhances the in vivo efficacy of MEK inhibitor therapy in uveal melanoma Faião-Flores, F., Emmons, M.F., Durante, M.A., Kinose, F., Saha, B., Fang, B., Koomen, J.M., Chellappan, S.P., Maria-Engler, S.S., Rix, U., Licht, J.D., Harbour, J.W., Smalley, K.S.M.: *Clin Cancer Res.* 2019 Sep 15;25(18):5686-5701. doi: 10.1158/1078-0432.CCR-18-3382. Epub 2019 Jun 21. PMID: 31227503

Decitabine limits escape from MEK inhibition in uveal melanoma. Goncalves, J., Faião-Flores, F., Emmons, M.F., Aplin, A.E., Harbour, J.W., Licht, J.D., Wink, M., Smalley, K.S.M.: *Pigment Cell Melanoma Res.* 2020 May;33(3):507-514. doi: 10.1111/pcmr.12849. Epub 2019 Dec 6. PMID: 31758842

HDAC inhibitors: A promising partner for MEK inhibitors in uveal melanoma. Faião-Flores, F., Smalley, K.S.M.: *Melanoma Manag*. 2019 Dec 16;6(4): MMT29. doi: 10.2217/mmt-2019-0017 PMID: 31871618

Patents: None at the time of reporting

3. **Grant #:** 7BC08 Lymphodepletion-generated Myeloid Derived Suppressor Cells Decrease the Efficacy of Adoptive T-cell Therapy for Melanoma

   Principal Investigator: Shari Pilon-Thomas, PhD

   Organization: Moffitt Cancer Center

   Grant Progress Report: Adoptive T-cell Therapy (ACT) in combination with lympho-depleting chemotherapy is an effective strategy to induce the eradication of tumors, providing long-term regression in cancer patients. Despite the establishment of a favorable immune milieu for ACT, lymphodepletion concomitantly promotes immunosuppress ion during the course of endogenous immune recovery. Here the team has identified that lymphodepleting chemotherapy initiates the mobilization of hematopoietic progenitor cells that differentiate to immunosuppressive myeloid

cells, leading to a dramatic increase of peripheral Myeloid Derived Suppressor Cells (MDSCs). In melanoma and lung cancer patients, MDSCs rapidly expanded in the periphery within one week after completion of a lymphodepleting regimen and infusion of autologous Tumor Infiltrating Lymphocytes (TIL). This expansion was associated with disease progression, poor survival and reduced TIL persistence in melanoma patients. Our team demonstrated lymphodepleting chemotherapy induced the production of IL-6 which regulated the survival and immunosuppressive capacity of post-lymphodepletion MDSCs genetic abrogation or therapeutic inhibition of IL-6 differentiation signals in mouse models reduced MDSC survival and their ability to suppress T cells, leading to enhanced host survival and reduced tumor growth in mice that received ACT. Thus, the expansion of MDSCs in response to lymphodepleting chemotherapy may contribute to ACT failure and targeting myeloidmediated immunosuppression may support anti-tumor immune responses.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

Bankhead-Coley Cancer Biomedical Research Program
Appendix J
Fiscal Year 2019-2020 Completed Grants
Funding Fiscal Year 2015-2016

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
6BC02	University of Miami	Anthony Capobianco, PhD	$1,343,732.00	$1,343,732.00	$0.00	4/20/2016	9/30/2019	No	No	No
6BC05	Mayo Clinic	Aubrey Thompson, PhD	$1,064,624.44	$1,058,487.03	$6,137.41	4/12/2016	9/30/2019	No	Yes	Yes
6BC07	University of Florida	Michael Kladde, PhD	$681,887.44	$681,575.69	$311.75	3/31/2016	6/30/2016	Yes	No	No

1. **Grant#:** 6BC02 Lead Optimization and Preclinical Evaluation of Small Molecule Inhibitors of Notch Transcriptional Activation

   Principal Investigator: Anthony J. Capobianco, PhD

   Organization: University of Miami Miller School of Medicine

   Grant Progress Report: In summary, the data shown below demonstrate that the structure-activity relationship (SAR) study performed on analogs of 1-134-83 have led to the identification of the most potent compound in our studies, which was named NADI-351. Our team determined the pharmacokinetic profile of NADI-351, as well as its glutathione reactivity, which is related to its Michael acceptor ability. The data indicate that the compound is stable in mice plasma and does not exhibit Michael acceptor reactivity, which has been perceived as a liability in rhodanine-bearing compounds. In addition, the strategy followed to improve stability of NADI-260 to obtain NADI-351 was successful. Notably, NADI-351 is orally bioavailable and it does not exert gastrointestinal toxicity, as exhibited by y-secretase inhibitors. Compound NADI-351 inhibits tumor growth in a patient derived xenograft model and the data suggest that NADI-351 selectively inhibits Notch1 recruitment to the Notch ternary complex and inhibits Notch target genes expression, which results in inhibition of Maml1 recruitment to the complex, providing insight into the mechanism of action of NADI-351 and its analogs. As part of the comprehensive evaluation of the lead candidate NADI-351, our team will evaluate the effect of compound NADI-351 and its respective control (vehicle) on global gene expression through RNA sequence analysis (RNA-seq) in OE33 cells (esophageal adenocarcinoma). For comparison, our team has successfully used siRNA for the knockdown of Notch paralogs (Notch1, Notch2, and Notch3, since OE33 cells do not express Notch4 paralog) and will also determine the effect of knockdown on global gene expression by RNA-seq. The RNA-seq measurements will be performed at the SCCC Genetics Core at the University of Miami.

   The SAR strategy in combination with molecular docking simulations using the Molecular Operating Environment (MOE) software have given the team insight into the putative binding pocket of NADI-351 in the Notch ternary complex. Therefore, our team will determine the affinity of NADI-351 to proteins in the Notch ternary complex and obtain insight into the binding pocket using SPR. Our team will continue collaborating with Dr. Kovall from the University of Cincinnati to elucidate the crystal structure of the protein-compound complexes and Dr. Kovall's group is also involved in determining the key biomolecular interactions involved in the inhibition of Notch transcriptional activation. As part of the team's optimization process, Dr. Kovall's group have obtained new CSL-ANK fusion crystals from Hauptman-Woodward high-throughput screening. These new fusion crystals still need to be optimized and the team will check for diffraction before soaking with the selected compounds, including NADI-351. One graduate student is receiving training under the direction of Dr. Kovall.

   Since the pharmacokinetic study of NADI-351 indicate a good oral bioavailability of the compounds, our team will use the pilot study on xenografts corresponding to various types of Notch-dependent cancers to identify 2-4 models of cancers to evaluate the effect of NADI-351.These models will be used to evaluate the effect of the compounds on xenografts and PDX models of cancer, including oral administration of the compound. Our team will complete the characterization of excised tumor samples from mice treated with NADI-351, which includes immunohistochemistry. The results from this study will help the team to determine the mechanism of action of NADI-351 and its analogs, as well as to validate NADI-351 as a small molecule inhibitor of the Notch transcriptional activation complex for further development.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

2. **Grant #:** 6BC05 Predictive Markers of HER2-Targeted Therapy

Principal Investigator: E. Aubrey Thompson, PhD

Organization: Mayo Clinic Florida

Grant Progress Report: Broadly speaking, our work has focused on identifying features that are associated with clinical outcome following adjuvant trastuzumab therapy in early stage HER2-positive (HER2+) breast cancer. Our work has identified major features relating to chromosomal integrity and tumor heterogeneity. From a clinical standpoint, our analysis of risk of recurrence in Estrogen Receptor positive (HER2+/ER+) and negative (HER2+/ER-) has the potential to prevent overtreatment of about half of all HER2+ patients. Although not within the original aims of this project, the team has begun to take advantage of rapidly emerging spatial biology technology, which will enable our staff, in the future, to analyze, in detail, tumor cell-immune cell interactions and the extent to which such interactions are linked to clinical outcome. Spatial biology will dominate breast cancer research for the next few years. Traditionally, the role of the immune system in breast cancer therapy has been estimated by measuring lymphocytes that have infiltrated the tumor stroma, the so-called Tumor Infiltrating Lymphocytes (TlLs). As charter members of the International Working Group for Breast Cancer Immune Biomarkers, our team has played a significant role in standardizing this technology. Data generated under 6BC05 are currently being analyzed as part of a collaborative meta-analysis of TlLs in HER2+ breast tumors. However, conventional histo-pathological evaluation of Tl Ls, no matter how well controlled and carefully performed, will not ultimately provide the depth of information that our team will require to understand the immune-biology of breast cancer. What is needed is the ability to precisely quantify the number, types, activities, and locations of immune cell subtypes with individual breast tumors. This technology is now available. Our team has spent the last few months of the 6BC05 funding period beta testing the NanoString GeoMX Digital Spatial Profiling (DSP) platform. Our team currently have the ability to measure the abundance of 55 key immune function proteins in an operator-designated spatial context using formalin-fixed, paraffin-embedded archival samples. Our team has begun to identify heretofore unappreciated immune features that are linked to clinical outcome. Studies of this sort, which spun out of the last few months of the Bankhead Coley grant, will define our future directions.

Follow-on Funding: Breast Cancer Research Foundation; Spatial Biology of Breast Cancer; Dr. E. Aubrey Thompson; $250,000.

Collaborations: Our team has established major collaborative efforts with the following individuals and institutions: Roberto Salgado, Richard Gray, Sherene Loi, meta-analysis of TlLs in HER2+ breast cancer Katherine Pogue-Geille, NSABP, molecular signatures that predict outcome in HER2+ breast cancer Charles M. Perou, UNC, molecular signatures that predict outcome after trastuzumab

Richard Baehner, Genomic Health, TILs in HER2+ breast cancer Sunil Badve, U. Illinois, TILs in HER2+ breast cancer

Douglas Hinefeld, Sarah Warren, Heather Ann Brauer, David Henderson, NanoString, molecular signatures associated with outcome in breast cancer

Saranya Chumsri, Mayo Clinic Florida, clinical breast cancer outcome

Jodi Carter, Mayo Clinic Rochester, breast cancer pathology and histology

Journals: Effects of Age and Immune Landscape on Outcome in HER2+ Postive Breast Cancer in the NCCTG N9831 (Alliance) and NSABP B-31 (NRG) Trials. Chumsri, S, DJ, Li Z, Pogue-Geile KL, Soyano- Muller AE, Mashadi-Hossein A, Warren S, Lou Y, Colon-Otero G, Knutson KL, Perez EA, Moreno-Aspitia A, Thompson EA. Clinical Cancer Research. 2019.

Functional Annotation of ESR1 Gene Fusions in Estrogen Receptor-Positive Breast Cancer. Lei JT, Shao J, Zhang J, Iglesia M, Chan DW, Cao J, Anurag M, Singh P, He X, Kosaka Y, Matsunuma R, Crowder R, Hoog J, Phommaly C, Goncalves R, Ramalho S, Peres RMR, Punturi N, Schmidt C, Bartram A, Jou E, Devarakonda V, Holloway KR, Lai WV, Hampton O, Rogers A, Tobias E, Parikh PA, Davies SR, Li S, Ma CX, Suman VJ, Hunt KK, Watson MA, Hoadley KA, Thompson EA, Chen X, Kavuri SM, Greighton CJ, Maher CA, Perou CM, Haricharan S, Ellis MJ. Cell Reports. 2018.

Chromoanasynthesis is a common mechanism that leads to ERBB2 amplifications in a cohort of early stage HER2+ breast cancer samples. Vasmatzis, G, Wang, X, Smadbeck, JB, Murphy, SJ, Geiersbach, KB, Johnson, SH, Gaitatzes, AG, Asmann, YW, Kosari, F, Borad, MJ, Serie, DJ, Mclaughlin, SA, Kachergus, JM, Necela, BM, Thompson, FA, BMC Cancer, 2018.

Patents: None at the time of reporting

3. Grant #: 6BC07 Temporal Epigenetic Mechanisms in Breast Cancer Oncogenesis

Principal Investigator: Michael P. Kladde, PhD

Organization: University of Florida

Grant Progress Report: O single-molecule Methylation Accessibility Protocol of Individual Templates (MAPit) methylation foot-printing of immortalized breast epithelial cells (parental M1); 2) discovered intriguing biallelic pattern of methylation and chromatin accessibility at the promoter of S/M2, encoding a breast cancer tumor suppressor; 3) established a collaboration with Kapa Biosystems (Roche) to perform targeted MAPit of 5.5 million CG sites and 12; 4) developed MAPit differential methylation analysis pipeline (DMAP), i.e., capable of simultaneous bioinformatic analysis of CG and GC methylation; 5) validated antibodies for genome-wide assessment of histone marks using chromatin immunoprecipitation coupled with exonuclease treatment (ChlPexo); 6) optimized conditions for performing chromatin accessibility by assay for transposase-accessible chromatin using sequencing (ATAC-seq); and 7) obtained lentiviral vector with the reverse tetracycline repressor for construction of stable M1 lines for doxycycline-inducible HRAS-G12V expression.

We successfully recruited and hired (July 28, 2017) Biological Scientist II (technician), Marie-Pierre Gauthier, MS, who has ten years' experience in gene regulation and genomics; 2) hired (May 16, 2017) Postdoctoral Associate, Rosha Poudyal, PhD, who trained in the Principal Investigator's laboratory; 3) obtained and expanded new, viable stocks of the M1-M4 cell line series that models HRAS-G12V-induced breast cancer progression; 4) obtained high-quality MAPit samples for lines M2-M4 in duplicate; 5) developed new protocol and data analysis pipeline for sequencing long bisulfite-converted amplicons on Pacific Biosciences (PacBio) sequencers (RSII and Sequel); 6) made substantial improvements in the computational utility and speed of DMAP, yielding DMAP2 for analysis of large MAPit datasets; 7) improved our MAPit assay for capture of targeted sequences; 8) performed duplicate, high-quality ATAC-seq for M1-M4 and developed a custom pipeline for calling differentially accessible regions; 9) completed all six pairwise M1-M4 ATAC-seq dataset comparisons to identify differentially accessible regions; 10) in the M2 (pre-malignant) vs. M1 (parental) comparison, identified 94 candidate RNA polymerase II gene promoters with at least 4-fold decrease in accessibility; and 11) constructed lentivirus for doxycycline-inducible expression of HRAS-G12V.

Our team successfully recruited and hired (September 4, 2018) Postdoctoral Associate, Anqi Wang, PhD, who has extensive previous training in epigenetics and replaced Guanyang Zhang, PhD, who accepted another position. Our team has also successfully recruited and hired (January 1, 2019) Postdoctoral Associate, Jason Brant, PhD, who has extensive previous training in epigenetics as well as bioinformatics and made substantial improvements to our bioinformatics pipeline, mainly in data normalization and nearest genomic feature assignments, for calling differentially accessible regions in chromatin. Our team repeated MAPit on line M1 as one replicate did not pass quality control and completed analysis of a positive control promoter and six candidate promoters across all four M series cells in triplicate using PacBio sequencing of long bisulfite converted amplicons, showing progressive increases in DNA methylation and, conversely, decreased chromatin accessibility. Our team performed MAPit coupled with sequence capture and epigenetic analysis of a 'giant' number of CG and GC sites (SeqCap Epi CpGiant), hereafter, MAPit-CpGiant, of all 12 MAPit samples and developed a bioinformatics pipeline to analyze large MAPit-CpGiant datasets, including novel mean to normalize the data, sequenced 12 MAPit-CpGiant libraries and analyzed the results, identifying many additional regions that exhibit epigenetic changes in response to introduction of oncogenic HRAS; developed and optimized precision, flap-enabled next-generation capture (FENGC) of targeted sequences from MAPit samples (MAPit-FENGC); and identified prominent epigenetic alterations in response to oncogenic HRAS in pathways that govern cell signaling, apoptosis, and epithelial-to-mesenchymal transition. Our team has generated three independent time-course experiments by transducing parental M1 cells with oncogenic HRAS (HRAS-G12V) to determine the spatiotemporal molecular events that occur during transformation-dependent epigenetic silencing over time; and amplified and performed PacBio sequencing of bisulfite-converted amplicons from three time-course replicates, validating additional loci that are open and unmethylated in M1 cells and closed and hypermethylated in M2 cells.

Follow-on Funding: None at the time of reporting

Collaborations: Nancy Nabilsi, PhD, Senior Applications Scientist, Kapa Biosystems (Roche) to merge MAPit with SeqCap Epi CpGiant targeted enrichment of 5.5 million CpG sites for bisulfite sequencing, creating a new technology MAPit-CpGiant.

Patrick J. Concannon, Director, University of Florida Genetics Institute, Professor of Pathology, Immunology, and Laboratory Medicine to identify epigenomic changes induced by low-dose radiation in Brassica rapa L., a plant species in the mustard and cabbage family.

Mark A. Atkinson, PhD, Director, University of Florida Diabetes Institute, Professor of Pathology, Immunology, and Laboratory Medicine to identify changes in DNA methylation and chromatin accessibility associated with GWAS mutations in type 1 diabetic patients.

Nagi Ayad, PhD, Associate Professor of Psychiatry and Behavioral Sciences, Sylvester Comprehensive Cancer Center, University of Miami Health Center, investigating the epigenetic basis of chemotherapy resistance in glioblastoma.

Rhonda Bacher, PhD, Assistant Professor of Biostatistics, University of Florida, Gainesville to develop and apply statistical methods to analyze MAPit datasets.

James L. Van Etten, PhD, William Allington Distinguished Professor of Plant Pathology, University of Nebraska, Lincoln to identify additional DNA methyltransferases to increase MAPit chromatin mapping resolution.

Lu Bai, PhD, Associate Professor of Biochemistry and Molecular Biology and Physics, The Pennsylvania State University, University Park, PA to use long-read MAPit sequencing to define rules for transcription factor binding.

Matthew D. Scharff, PhD, Distinguished Professor of Cell Biology, Albert Einstein College of Medicine, NY to use MAPit to determine how chromatin structure regulates antibody combinatorial diversity in mammalian B cells.

Mansour Mohamadzadeh, PhD, Professor of Infectious Diseases and Immunology, University of Florida, Gainesville to determine how vitamin B12 governs epigenetic regulation of mouse ileal epithelial cells.

Thomas A. Clanton, PhD, BK and Betty Stevens Professor of Applied Physiology and Kinesiology, University of Florida, Gainesville to identify epigenetic markers in blood monocytes as well as skeletal and cardiac muscle in a mouse model of exertional heat stroke.

Olga Guryanova, MD, PhD, Assistant Professor of Pharmacology, University of Florida, Gainesville to determine genome-wide changes in chromatin accessibility and DNA methylation during progression of leukemias with DNMT3A mutations.

Journals: None at the time of reporting

Patents: Determination of Methylation State and Chromatin Structure of Target Genetic Loci
Inventors: Michael P. Kladde, PhD, Nancy H. Nabilsi, PhD, and Carolina E. Pardo, PhD
Assignee: The University of Florida; patent No. 10,435,740 issued October 8, 2019

James and Esther King Biomedical Research Program
Appendix K
Fiscal Year 2020-2021 Newly Awarded Active Grants

Grant #	Organization	Principal Investigator	Award Amount	Life to Date	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
20K01	Florida State University	Pradeep Bhide, PhD	$626,708	$0.00	$626,708	6/11/20	5/31/23	No	No	No
20K02	Mayo Clinic Jacksonville	Debabrata Mukhopadhyay, PhD	$626,708	$34,820	591,888	5/14/20	4/30/23	No	No	No
20K03	Scripps Florida	Thomas Kodadek, PhD	$250,680	$0.00	$250,680	6/5/20	5/31/23	No	No	No
20K04	University of Central Florida	Ulas Bagci, PhD	$1,112,880	$61,826	$1,051,054	6/16/20	4/30/23	No	No	No
20K05	University of Florida	Terence E. Ryan, PhD	$626,710	$0.00	$626,710	6/12/20	5/31/23	No	No	No
20K06	University of Florida	Gilbert R. Upchurch, Jr., MD	$626,708	$0.00	$626,708	6/11/20	5/31/23	No	No	No
20K07	University of Florida	Daiqing Liao, PhD	$626,708	$0.00	$626,708	6/11/20	5/31/23	No	No	No
20K08	University of Florida	Dorian K. Rose, PhD	$688,940	$0.00	$688,940	6/12/20	5/31/25	No	No	No
20K09	University of Miami	Ami P. Raval, PhD, MSPH	$626,710	$0.00	$626,710	6/5/20	5/31/23	No	No	No
20K10	University of Miami	Taghrid Asfar, MD, MSPH	$1,253,415	$0.00	$1,253,415	6/18/20	5/31/25	No	No	No
20K11	University of Miami	Miguel Perez-Pinzon, PhD	$626,708	$0.00	$626,708	6/9/20	5/31/23	No	No	No

1. **Grant #:** 20K01 Nicotine, Germ Cells and Neurodevelopmental Disorders

   Principal Investigator: Pradeep Bhide, PhD

   Organization: Florida State University

   Abstract of Proposed Research: Use of tobacco products is a well-known risk factor for the development of cancer and cardiovascular disease. However, beyond the obvious and serious issues of nicotine addiction, the consequences of nicotine use for cognitive function especially for neuropsychiatric conditions in children and adolescents, is less well understood. Nicotine use by pregnant women is associated with significant increase in risk of developmental neurobehavioral disorders including Attention Deficit Hyperactivity Disorder (ADHD) in their children. In the U.S., approximately 7% of pregnant women smoke cigarettes, and the prevalence increases to almost 16% during the initial postpartum period. With the availability of electronic nicotine delivery systems, the incidence of nicotine use has increased significantly. Perhaps more alarming is the recent discovery that nicotine's impact on the brain and behavior may transcend the directly exposed individual or generation and may be evident in multiple generations descending from the directly exposed individual. This "transgenerational transmission" of nicotine's effects on the brain and behavior brings into sharp focus nicotine's effects on germ cells. Animal models show that the transgenerational heritability of nicotine's effects does not follow the classic Mendelian principles, suggesting epigenetic modification of germ cells rather than changes in DeoxyriboNucleic Acid (DNA) sequence (i.e. mutations) as a plausible mechanism of heritability. We are among the first to develop and use a pre-clinical (mouse) model to examine transgenerational transmission of nicotine-induced neurodevelopmental phenotypes. Our data shows that nicotine produces epigenetic modification of germ cell DNA; alters neurotransmitter signaling, neurogenesis and neuronal migration in the brain; and interferes with multiple cognitive domains. The changes in the brain and behavior are consistent with the pathophysiology and symptoms of neurodevelopmental disorders such as ADHD and autism, whereas the epigenetic changes in germ cells are consistent with transgenerational transmission of the neuropsychiatric phenotypes. Thus, a framework for defining a novel link between nicotine, neurodevelopmental disorders and germ cell epigenetics is emerging. However, the different elements in this framework namely, the brain cells, cognitive domains and germ cells have not been integrated into a comprehensive mechanistic matrix yet. To address this gap, we propose to examine cellular and molecular mechanisms that link nicotine's effects on the brain and behavior on the one hand with its effects on germ cells on the other. Specifically, we will test the hypothesis that prenatal and/or postnatal nicotine exposure produces significant alterations in neurotransmitter signaling, neurogenesis, and neuronal migration and produces epigenetic modifications in germ cells. We will also examine nicotine -induced cognitive changes in adolescence and adulthood to determine if they are consistent with the cellular and molecular changes observed in the brain. Finally, we will determine if the epigenetic changes in germ cells are consistent with a potential mechanism for transgenerational transmission of the nicotine-induced neurobehavioral phenotypes. We will use a mouse model and state-of-the-art technology already in use in our laboratories to examine nicotine-induced cellular, molecular and behavioral changes including epigenetic modifications of germ cells.

   Follow-on Funding: None at the time of reporting

   Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

2. **Grant #:** 20K02 A Novel Therapy for Advanced Drug-Resistant Lung Cancer with an Emphasis on Smoking-Induced Exacerbation

Principal Investigator: Debabrata Mukhopadhyay, PhD

Organization: Mayo Clinic

Abstract of Proposed Research: Lung cancer remains the leading cause of cancer related deaths in United States and accounts for about 25% of all cancer related deaths. Approximately 228,150 new diagnoses (116,440 in men and 111,710 in women) and 142,670 deaths (76,650 in men and 66,020 in women) are estimated from lung cancer among US population in 2019. Among them, the state of Florida will have approximately 18,560 new diagnoses and 10,880 deaths in 2019. Cigarette smoking is associated with more than 80% of lung cancers in United States. Cigarette smoke contains numerous carcinogens, including polycyclic aromatic hydrocarbons (PAH) and mutagenic nicotine metabolites. Cigarette smoke can enhance oncogenic addiction to cMET and impart resistance towards epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKI). A considerably large subgroup of lung cancer patients resistant to EGFR-TKIs exhibits cMET overexpression or amplification. A noticeable increase in the expression of C-X-C chemokine receptor 4 (CXCR4) was also detected in nicotine-treated lung cancer cells. CXCR4 is a G protein-coupled receptor that binds its ligand stromal cell derived factor 1 (SDF-1) also known as C-X-C chemokine ligand 12 (CXCL12). The CXCL12/CXCR4 axis has been implicated in several biological processes, including cell skeleton rearrangement, cell migration, and trafficking and homeostasis of immune cells. Cancer cells often hijack CXCL12/CXCR4 axis to promote their survival, growth, metastasis, chemo-resistance, and immune evasion. High CXCR4 expression exhibits significant correlation with lymph node metastasis, distant metastasis, tumor stage and overall survival in lung cancer patients. Interestingly, CXCR4 inhibition has been shown to regulate epithelial mesenchymal transition of lung cancer cells via Hippo-yes-associated protein (YAP) signaling pathway. The team is collaborating with Prof. Dr. Hsu-Shan Huang (Taipei Medical University) who developed a novel small-molecule 6,6,6,6-tetraheterocyclic derivative (TC-N19, now NDT-19), that led to the degradation of both EGFR and cMET to override EGFR-TKI resistance in lung cancer cells. The Professor Huang's group showed that NDT-19 was able to suppress growth in both EGFR-TKI- sensitive and -resistant tumors without affecting mice body weight. NDT-19 also showed no toxicity towards normal lung cells and reticulocytes. In addition, our preliminary data suggest that NDT-19 treatment leads to the degradation of CXCR4 and downregulation of YAP in lung cancer cell lines. Taken together, NDT-19 is a first-of-its-class multi-target inhibitor demonstrating remarkable antitumor efficacy in lung cancer. In this regard, the central hypothesis of this application is that NDT-19, due to its efficient multi-target inhibition, will demonstrate strong antitumor efficacy via direct effect on tumor cells as well as leveraging tumor microenvironment- mediated indirect mechanisms such as inhibition of angiogenesis and enhanced antitumor immunity and can improve the clinical outcome of the patient of lung cancer, in particular with the smoking related diseases. In this proposal, we intend to investigate the therapeutic efficiency of a liposomal formulation of NDT-19 (LNDT-19) in smoking-related lung cancer and elucidate the therapeutic mechanisms. We will investigate its effects on the tumor cells as well as tumor microenvironment. We expect to gather pertinent data to support

future investigations of LNDT-19 as an anticancer agent.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

3. **Grant #:** 20K03 Selective Proteolysis of p53 Missense Mutants

Principal Investigator: Thomas Kodadek, PhD

Organization: Scripps Florida

Abstract of Proposed Research: The transcription factor p53 plays a critical role in the development of cancer. p53 is a transcription factor that halts the progression of the cell cycle under conditions of genotoxic stress to allow repair of carcinogenic DNA damage. If the damage is too severe, p53 activates an apoptosis program. Missense mutations in p53 that inactivate the protein are found in more than 50% of all cancers and an even higher percentage of patients with aggressive carcinomas. It is the most commonly found mutation in tobacco- related cancers, especially in the lung. By far the most common cancer-driving point mutations in p53 are located within the DNA-binding domain (DBD). These mutations destabilize the domain thermally. It is misfolded at 37oC, and thus is unable to bind to DNA and unable to activate transcription. This would be bad enough, but the more serious problem is that the missense p53 mutants accumulate to very high levels in cancer cells and display both dominant negative behavior and toxic gain of functions that are strongly tumor promoting. A drug capable of blocking these functions of mutant p53 proteins would be transformative for cancer chemotherapy. We propose to develop a PROTAC (proteolysis targeting chimera) able to engage p53 missense mutants. PROTACs are chemical dimerizers capable of forcing a target protein and an E3 UBiquitin Ligase (Ubl) complex into proximity, resulting in poly-ubiquitylation and subsequent proteasome-mediated degradation of the target. PROTACs targeting many different proteins have been reported over the last few years, most of which contain existing ligands for the VHL or cereblon E3 ubiquitin ligases. Therefore, the seminal issue in developing a p53 missense mutant targeted PROTAC is identifying a ligand for the misfolded DBD of the mutant protein. Moreover, it is critical to find a ligand that cleanly distinguishes the mutant protein from wild type p53, since knocking down the level of functional p53 is obviously not a good idea. The team will screen novel DNA-encoded libraries developed recently in this laboratory to identify the appropriate ligand. These molecules will then be conjugated to an E3 ubiquitin ligase ligand to create a PROTAC that will be tested in various cancer cell lines. While this project is somewhat risky, involving a high-throughput screen against a novel target, the payoff would be enormous.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

4. **Grant #:** 20K04 Predicting Outcomes of Lung Cancer Therapy Through Explainable Deep Learning

   Principal Investigator: Ulas Bagci, PhD

   Organization: University of Central Florida (UCF)

   Abstract of Proposed Research: Radiation therapies have proven to offer significant treatment advantages to patients presenting with lung cancers. In all types of radiation therapy, the majority of the ionizing energy is focused on the target lesion and its immediate area with lower damage to nearby vital organs. However, some patients still develop complications in the surrounding tissue as a side effect of radiation therapy treatments. This innovative project will conduct a retrospective study across two cohorts of lung cancer patients who received Radiation therapy treatment (including proton therapy). Imaging datasets and clinical documentation created during the treatment process will be used to develop an explainable artificial intelligence (AI) system that functions as an early predictor for treatment outcomes. Many AI systems currently developed today can produce accurate predictions but cannot be used in clinical settings because the reasons for the prediction results are not easily understood, namely black box models, making these systems difficult for regulators to review and approve for clinical use. In contrast, the project will develop an explainable AI system and evaluate its effectiveness using cohorts of patient data curated for this project. To achieve this overall goal, the team will first collect and process all patient imaging and treatment record documentation using an automated, repeatable process. Then, advanced techniques will generate an explainable and highly accurate system trained to predict clinical outcomes for each individual patient. The team will test and evaluate our predictor against a blind dataset as well as an open dataset with the hypothesis that new algorithms will be more accurate, efficient, and explainable than the conventional radiomics approaches. The blind dataset will be supplied, at no cost, by an existing effort already underway at the Frederick National Laboratory for Cancer Research (FNLCR). The second cohort dataset will be provided by the Radiation Therapy Center at Orlando Health University of Florida Cancer Center in Orlando, Florida. All algorithm development will be performed by doctoral students, postdoctoral fellows from UCF, and KnowledgeVis, LLC, a Florida-based small business specializing in medical imaging research. Methods development and results validation will be conducted under the supervision of PI Bagci of the University of Florida (UCF), Co-PI Kelly of Orlando Health, and Co-PI Lisle of KnowledgeVis, LLC.

   Our team believes this strong collaboration between all Florida-based entities is uniquely capable of developing prediction tools for eventual use by clinicians. If successful, this research can improve the outcome of Radiation therapy patients suffering with multiple types of lung cancer. The team expects the outcome of this project to be strong early results, suitable for applying to continue this research with a National Institutes of Health funded study.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

5. **Grant #:** 20K05 Role of the Aryl Hydrocarbon Receptor in Tobacco Smoke-Induced Skeletal Muscle Atrophy

   Principal Investigator: Terence Ryan, PhD

   Organization: University of Florida

   Abstract of Proposed Research: Low muscle mass and strength are independently associated with an increased risk of all-cause and cardiovascular disease mortality. Unfortunately, muscle mass declines with age leading to a loss of muscle strength. Smokers experience greater muscle loss (atrophy) than age-matched non-smokers. In fact, smokers that develop chronic obstructive pulmonary disease (COPD) display severe muscle atrophy leading to impaired mobility and a greater risk of death. Our preliminary results show that COPD patients with severe muscle atrophy have a large number of muscle fibers that have lost their connection to the motoneurons which impairs the ability to initiate muscle contraction. The team observed the same loss of muscle-motoneuron connections in mice exposed to tobacco smoke. These observations suggest the loss of muscle-motoneuron connection and muscle atrophy contribute to impaired muscle function in smokers. However, little is known about the mechanisms underlying these events, which presents a major barrier for the development of therapies to improve tobacco-related health outcomes. With a composition of ~4000 different chemicals, tobacco smoke is the most complex and least understood risk factor for mortality. Many of these chemicals bind to and activate the Aryl Hydrocarbon Receptor (AHR). In the lung, activation of the AHR upregulates detoxifying pathways that help degrade some of these chemicals to limit harm. However, novel discoveries from our lab show that tobacco smoke mediated AHR activation in skeletal muscle is harmful and causes muscle atrophy. Preliminary experiments show mice genetically modified to lack the AHR are protected from the adverse impact of tobacco smoke on the muscle fiber-motoneuron connections. Moreover, the team found that chronic activation of the AHR either genetically or through tobacco smoke exposure results in impaired function of mitochondria, which are structures in cells that generate energy and regulate muscle atrophy. Specifically, tobacco smoke exposure increased generation of reactive oxygen species (ROS) by mitochondria which are known to drive muscle atrophy. Importantly, genetic knockdown or pharmaceutical inhibition of the AHR were found to prevent mitochondrial impairments and muscle atrophy in muscle cells. Building on these exciting discoveries, this application will test the hypothesis that chronic activation of the AHR by tobacco smoke causes muscle atrophy. To accomplish this goal, we will utilize genetically modified mice that lack the AHR in skeletal muscle only and pharmacologic AHR inhibitors to determine if blocking AHR signaling in muscle improves muscle size and function in mice exposed to tobacco smoke. Importantly, the team will perform these studies using both preventative (given prior to tobacco smoke exposure) and rehabilitative (given after chronic tobacco smoke exposure) in order to achieve high clinical relevance. Finally, to further isolate

the role of the AHR, the team will examine if expression of a mutant form of the AHR which is always active (even without tobacco smoke) is sufficient to increase mitochondrial ROS and cause muscle atrophy. Collectively, these experiments are expected to reveal a novel mechanism linking tobacco smoke to muscle atrophy through chronic activation of the AHR. These studies will form the foundation for future therapeutic development aimed to manipulate the AHR pathway to prevent/treat muscle atrophy which may reduce morbidity and mortality in smokers.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

6. **Grant #:** 20K06 Role of Myeloid-Derived Suppressor Cells in Aortic Aneurysms and Rupture

Principal Investigator: Gilbert Upchurch Jr., MD

Organization: University of Florida

Abstract of Proposed Research: Aortic aneurysms affect 5% of the population aged >65 years, with the incidence 3-5 times higher in smokers than in non-smokers. Furthermore, tobacco smoke increases the rate of aortic expansion and the risk of aortic rupture. However, how tobacco use influences aortic aneurysms and rupture has not been addressed. In this proposal, propose that tobacco smoking and age alter epigenetics-regulated myeloid-derived suppressor cell (MDSC) numbers and function that exacerbate inflammation and vascular remodeling during abdominal aortic aneurysm (AAA) formation and rupture. Smoking drives epigenetic changes (differences in gene expression that are not the result of changes or mutations in DNA sequence) in numerous cell types, and epigenetics have been demonstrated to play a role in pathological MDSC responses. We propose two hypotheses: (1) older, smoking adult humans have a persistent myelodyscrasia over time resulting in increased MDSC numbers with predominantly inflammatory phenotypes; and (2) an altered myelopoietic response to chronic smoking is secondary to unique MDSC epigenetic alterations. The team will obtain human aortic tissue and blood samples from 25 smoking and 25 non-smoking patients with AAA, as well as conduct experimental murine model studies using an elastase-treatment AAA model, as well as the recently described novel aortic rupture model, to investigate immunological regulatory function of MDSCs. To test our hypotheses, circulating and resident human G-MDSCs (CD11b+CD33+HLA- DRdimCD14-CD15+) and M-MDSCs (CD11b+CD33+HLA-DRdimCD14+) from blood and aortic tissue samples of smoking and non-smoking AAA patients will be isolated by fluorescent cell sorting. Subsequently, MDSCs will undergo epigenetic and functional analyses i.e. MAPit-CpGiant and MAPit-patch analysis (methylation-based foot printing followed by targeted bisulfite sequencing) to quantitate chromatin accessibility and DNA methylation on a substantial number of targeted loci simultaneously. In addition, highly enriched G- and M- MDSCs will undergo single cell RNAseq analysis to identify both inflammatory and immunosuppressive signatures. Using the murine AAA and aortic rupture models, we will analyze the role of MDSCs on increased AAA formation due to the deleterious effects of repeated exposure to nicotine, the major toxic in tobacco

smoke. To test this hypothesis, the team will conduct the same studies (nicotine treatment followed by elastase -treatment) in mice that cannot expand their MDSCs (crelox Lyz2-cebpb null) or cannot undergo emergency myelopoiesis (crelox VAV-rela null) compared to C57BL6 mice. The sequential expression and infiltration of MDSCs in blood and aortic tissue will be evaluated in these mice and associated with aortic diameter, inflammation (IL-1b, IL-17, HMGB1, IL-10, TGF-b1 expression), vascular remodeling (MMP2 and -9 activity) and immunohistology (M1/M2 macrophages and neutrophil infiltration, smooth muscle cell integrity and elastin degradation). The mechanistic crosstalk between MDSCs and CD11b+/F4/80+ macrophages (to enhance IL-1b, HMGB1, and IL-10 expression), Ly6G+ PMNs (to promote neutrophil extracellular trap (NET) formation), and aortic smooth muscle cells (to increase TGF-b1 signaling), with/without varying doses of nicotine will be deciphered by *in vitro* co-culture studies. Overall, our proposed studies are focused at unraveling the previously unknown role of MDSCs in the pathogenesis of smoking-induced aortic aneurysms and rupture.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

7. **Grant #:** 20K07 Molecular Mechanisms and Pharmacologic Targeting of Lipogenesis in Breast Cancer

   Principal Investigator: Daiqing Liao, PhD

   Organization: University of Florida

   Abstract of Proposed Research: Breast cancer is the most frequently diagnosed cancer type and the second leading cause of cancer-related mortalities in women in the United States. Although endocrine therapies such as tamoxifen and letrozole that block the Estrogen Receptor a (ER) pathway in breast tumors remain the main options for clinical treatment of ER+ breast cancer, only about 50% of patients with ER+ breast cancer show response to endocrine therapies. For Human Epidermal Growth Factor Receptor 2 (HER2) enriched subtype, only about 35% of HER2-enriched tumors show response to HER2-targeted therapies (e.g., trastuzumab and lapatinib). Triple-negative breast cancer (TNBC) accounts for 15 to 20% of all breast cancer cases. Although TNBC patients with preoperative chemotherapy exhibit higher rates of pathological complete response than patients with ER+ breast cancer, intrinsic resistance to chemotherapy is common. Furthermore, acquired resistance to standard-of-care therapies for all breast cancer subtypes is another major hurdle for durable treatment response. Therefore, there is a clear unmet medical need to discover and develop novel, safe and effective therapeutics for patients with advanced breast cancer that no longer responds to available therapies. The goal in this application is to define an oncogenic mechanism that promotes the production of lipids and cholesterol required for aggressive breast tumor growth and evaluate the safety and efficacy of a novel peptide targeting this mechanism for treating breast cancer. Aggressive tumor growth requires an increased supply of essential nutrients such as lipids and cholesterol for sustaining tumor cell proliferation. Uptake of dietary lipids is limited in nutrient -poor tumor microenvironment and intratumorally, necessitating an increase in

tumor-intrinsic production of lipids known as de novo lipogenesis. Statins, the cholesterol-lowering drugs, exhibit antiproliferative effects on cancer cells and clinical studies show that some breast cancer patients derive survival benefits with the use of statins. In preliminary studies, we have discovered a novel molecular mechanism that increases the production of lipid and cholesterol in cancer cells and drive tumor growth in vivo. Significantly, our preliminary studies indicate that a short polypeptide termed SIM2 could effectively block lipid production and restrict tumor growth. SIM2 administration in vivo was well tolerated in preclinical animal studies. Thus, this novel agent may have therapeutic potential for treating patients with advanced breast cancer.

SIM2 has a molecular weight similar to small molecule drug compounds; thus, it is suitable for various systemic treatments, such as via oral administration. Because SIM2 has a defined target within tumor cells, it is less likely to hit other targets in the human body, and thus is expected to be safe. Although our preliminary studies show that SIM2 is very well tolerated and not toxic in animal experiments, the safety and pharmacologic properties of SIM2 will be further rigorously assessed to ensure that it will be safe and suitable for clinical applications for treating cancer patients. In summary, our studies in this proposal may define a novel mechanism that drives breast cancer growth and validate a potential new treatment for patients with advanced breast cancer that does not respond to available therapeutic options.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

8. **Grant #:** 20K08 Augmenting a Post-Stroke Wellness Program with Respiratory Muscle Training: A Randomized Controlled Trial

   Principal Investigator: Dorian Rose, PhD

   Organization: University of Florida

   Abstract of Proposed Research: Stroke is a devastating neurological injury, the leading cause of disability in the United States, and in 25% of cases directly attributable to cigarette smoking. Although arm and leg weakness are the most obvious sources of impaired motor function and persistent disability, many stroke survivors also experience respiratory muscle weakness, which may impede post-stroke rehabilitation and increase risk of health complications. Despite clinical practice guidelines recommending smoking cessation, 18-35% of stroke survivors continue to smoke, compounding the respiratory compromise. Individuals post-stroke with a smoking history are particularly vulnerable to pulmonary complications, recurrent stroke, and increased risk of death. On the other hand, individuals ‘post-stroke who undergo Respiratory Muscle Training (RMT) increase not only their respiratory strength but experience improved lung health and functional mobility. Despite these encouraging findings, two crucial questions remain:will RMT deliver an additive benefit to individuals in a post-stroke supervised exercise program, and will individuals with a smoking history respond differently to RMT than non-smokers?

The central hypothesis guiding this proposal is that a stroke wellness program augmented with respiratory muscle training will confer an added rehabilitative benefit to chronic stroke survivors with a smoking history. The team plans to utilize a novel, community-based Stroke Wellness Program (SWP) tailored to maximizing an individual's functional and respiratory capabilities post-stroke. The SWP includes stretching, strengthening and cardiovascular exercises three times weekly for eight weeks and follows the progression guidelines recommended by the American Heart Association. The team will enroll adult, ambulatory individuals at least 6 months post-stroke, and then randomize participants either to a standard care group that undergoes SWP, or an enhanced care group that receives RMT in addition to SWP. Rehabilitative benefits of the two treatment approaches in the smoking and non-smoking participants will be compared at baseline, at the 1-month mid-point and at the 2-month program endpoint, and then longitudinally for 1-year to track adverse health events. To test the central hypothesis, there are four specific aims: evaluate short- and long-term cardiovascular and pulmonary benefits of the programs, assess the effect of the interventions on walking and functional mobility as well as airway clearance function, contrast how these two approaches translate into community ambulation and participation in society, and investigate the interaction of smoking history and rehabilitation approach on the incidence of 1-year adverse health events.

This innovative work will provide the first controlled, empirical evidence concerning the rehabilitative effects of SWP and RMT according to smoking history. It may have profound implications on tailoring the exercise prescription to more comprehensively address the added functional and pulmonary health risks associated with smoking. The team is hopeful that knowledge gained from this project will promote cardiovascular and pulmonary health, restore independent functional mobility and life participation, and prevent respiratory complications for all individuals living with chronic stroke.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

9. Grant #: 20K09 Nicotine Alters Brain Metabolism and Exacerbates Ischemic Brain Damage

Principal Investigator: Ami Raval, MSPH, PhD

Organization: University of Miami

Abstract of Proposed Research: In Florida, 17% of women smoke cigarettes. Smoking related mortality accounts for an average loss of 14 years from a woman's life. Women smokers of reproductive age are more likely to use Oral Contraceptives (OC). The combination of cigarette smoking and OC increases women's risk for cerebral ischemia (a consequence of stroke or cardiac arrest) by 30 folds. Even though smoking is a black box warning on OC label, the sad reality is women continue to concurrently smoke cigarettes and use OC. Oral contraceptives and smoking derived Nicotine (N) are known to synergistically increase the risk and severity of cerebral ischemia in females. Although it has been known for some time that long-term use of OC and nicotine (OC+N) cause an increased risk of peripheral thrombus formation, the exact

mechanism by which a combination of OC+N causes cerebral ischemia is not clear. Additionally, the effect cessation of smoking has on reducing the severity of cerebral ischemia is not known. This proposal will address both these issues using an adult rat model of cerebral ischemia. The team's published studies demonstrate that the severity of ischemic hippocampal damage is far greater in female rats simultaneously exposed to OC than to N alone. Furthermore, the preliminary results demonstrate that nicotine withdrawal fails to decrease the severity of ischemic hippocampal damage in female rats previously exposed to OC+N. The data suggest that the synergistic effect of OC+N on the brain lasts longer than the effects of nicotine only. The long-term goal is to determine the mechanism(s) by which the combination of OC+N increases the risk of cerebral ischemia in females. The goal of this proposal is (1) to identify specific effects of OC+N on the hippocampus that are responsible for the increased severity of post-ischemic neuronal death and (2) to determine whether these effects are permanent after cessation of OC and nicotine delivery to female rats. One of the main causes of ischemic neuronal death is the loss of mitochondrial functions. Mitochondrial functions are regulated by mitochondrial Estrogen Receptor subtype beta (ERβ), and ERβ is required to protect the hippocampus from ischemic damage in females. The team's published study shows that ERβ regulated mitochondrial functions are impaired in the hippocampus of female rats exposed to OC+N. Importantly, mitochondrial dysfunction observed after OC+N is not observed in the group exposed to OC alone or nicotine alone. Taken together, these results emphasize that it is the combination of OC and nicotine that is important to study if the team wants to address the persistent severity of ischemic damage after OC+N or N withdrawal. The team hypothesize that the deleterious effects of OC+N on the hippocampus take longer to be mitigated after OC+N or N withdrawal because of the loss of ERβ mediated mitochondrial functions. Simultaneous usage of OC and nicotine poses unique and severe risks for chronic cerebrovascular diseases in women. The study will identify the consequences of nicotine withdrawal specific to OC exposed women, and ERβ could be a target for future translational research.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

10. Grant #: 20K10 Developing and Testing Water Pipe-Specific Health Warning Labels Targeting Young People in Florida

Principal Investigator: Taghrid Asfar, MD, MSPH

Organization: University of Miami

Abstract of Proposed Research: Waterpipe (WP) smoking (a.k.a. hookah) has become one of the leading tobacco use methods among youth in Florida. The impact of this dramatic rise is likely amplified by the mounting evidence of WP addictive and harmful nature, as well as the lag of policy response to it. Evidence suggests WP use leads to nicotine addiction, and increases the risk of lung cancer, heart and respiratory disease and exposure to secondhand smoke. The spread of WP use among youth has been fueled by a misperception of reduced - harm

compared to cigarettes. Health Warning Labels (HWLs) represent one of the most successful tobacco control strategies to communicate smoking-related risks, and studies have consistently shown that HWLs are associated with a decrease in smoking rates and smoking-related morbidity and mortality. Therefore, communicating WP risks to young people through HWLs has been identified as a priority by major health bodies in the US including the FDA. Currently, the FDA requires that WP tobacco packages have a textual HWL: "WARNING: This product contains nicotine. Nicotine is an addictive chemical." While this represents a good step, it is inadequate given the; 1- limited contact WP smokers have with tobacco packaging, 2- the established harm of WP smoking beyond tobacco (e.g., charcoal), and 3- the superior performance of pictorial HWLs over text-only ones. Therefore, pictorial WP-specific HWLs involving other WP components (e.g. device) are expected to be more effective in communicating risks to WP smokers. Using the Delphi method among tobacco control experts, our team has developed a set of 12 WP HWLs corresponding to four health themes (health risks and addiction, harm to others WP-specific harm, WP harm compared to cigarettes). Building on this work, and using a mixed-method approach incorporating qualitative and quantitative research, the team proposes to adapt the developed HWLs to young WP smokers in Florida and test them to answer an important policy question: Are pictorial HWLs on the WP device more effective than no-HWL (control) in increasing harm perception and intention to quit, and reducing smoking satisfaction, intensity, and exposure to toxicants? Accordingly, the team will recruit young (18-29 yrs) WP users to:

Conduct 6-8 mixed-gender focus groups ($n \approx 65$) to adapt the 12 HWLs to our local population of young adults in terms of risk communication, explore optimal placement and size of HWLs, and select the top 4 HWLs for testing in Aim 2.

Conduct a clinical lab experiment among 240 WP smokers categorized according to their use frequency into low vs. high to test the performance of the top 4 HWLs on the WP device compared to no HWL (control) on harm perception, intention to quit, puffing behavior, dependence (WP satisfaction, urge to smoke, withdrawal), and toxicant exposure (CO, nicotine, oxidative stress). The last session of study will be followed by a 3-month phone call assessment of change in harm perception and quit behavior.

Partner with Golin, Tobacco-Free Workgroup, and Truth Initiative to disseminate our research findings to increase awareness of WP harm among young adults and promote WP control through HWLs among policy makers in Florida.

Communicating WP risks through HWLs promises to reduce WP use and WP-related morbidly and mortality among young adults in Florida. This pioneer work will provide the first evidence-based WP HWLs to advance WP control in Florida, and a model for other states to respond to the WP epidemic.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

11. Grant #: 20K11 Strategies to Ameliorate Cognitive Decline Following Cerebral Ischemia in Nicotine-Exposed Rats

Principal Investigator: Miguel Perez-Pinzon, PhD

Organization: University of Miami - Miller School of Medicine

Abstract of Proposed Research: Millions of smokers are disabled as a result of stroke and ischemic stroke accounts for almost 85% of total stroke cases. Ischemic stroke kills more women than men. Currently, the only clinical therapies available for stroke are thrombolysis (tPA) and a newly developed mechanical endovascular recanalization, both of which have only limited applications in a small number of patients. Thus, the need for new neuroprotective therapies remains a high priority. Importantly, post-stroke cognitive decline remains a major issue and no treatment is available to enhance recovery for sub-acute and chronic stroke, emphasizing the need for new therapeutic developments. The vast body of literature suggests that physical exercise improves cognitive function and in a recent study staff demonstrated that post-stroke Physical Exercise (PE) significantly enhances cognitive recovery in rats. Studies have shown that the forced treadmill exercise can reduce ischemic brain damage and improve synaptic plasticity and learning and memory after stroke. Recent studies from the laboratory have shown that a regiment of treadmill PE is able to ameliorate cognitive deficits following Middle Cerebral Artery occlusion (MCAo; a well-established rat model of ischemic stroke) in young and 11-14-month-old male rats. However, aged rats were unable to run at the same speeds of the younger cohort, suggesting that as the rat's age increases, higher exercise intensities will be more difficult to attain. In addition, staff observed that in 12-month-old reproductive senescent female rats running at the same intensity as the corresponding age cohort of male rats did not exhibit cognitive recovery post MCAo. These data along with clinical data, suggests that to require ailing, elderly smoker MCAo patients to adhere to a chronic PE regimen presents a major obstacle. Therefore, the main goal of this project is to determine whether PE along with other therapies can ameliorate cognitive deficits in older cohorts of rats (male and female) that ensue from MCAo (plus nicotine).

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

James and Esther King Biomedical Research Program
Appendix L
Fiscal Year 2019-2020 Active Grants
Funding Year 2018-2019

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
9JK01	Florida State University	Gloria Salazar, PhD	$805,409	$201,351	$604,058	9/18/2019	9/30/2022	No	Yes	Pending
9JK02	H. Lee Moffitt Cancer Center	Shelley Tworoger, PhD	$504,838	$140,230	$364,608	8/15/2019	8/31/2022	No	No	No
9JK03	MCI/ Baptist Health South Florida	John P. Diaz, MD	$1,187,224	$118,722	$1,068,502	12/19/2019	12/31/2024	No	No	No
9JK04	University of Central Florida	Alicja Copik, PhD	$805,409	$67,116	$738,293	10/2/2019	9/2/2022	No	No	No
9JK05	University of Florida	Ramzi Salloum, PhD	$404,909	$33,741	$371,168	10/09/2019	9/30/2022	No	No	Yes
9JK06	University of Florida	Maria Zajac-Kaye, PhD	$805,409	$67,116	$738,293	9/18/2019	9/30/2022	Yes	No	No
9JK07	University of Miami	Vikas Dudeja, MD	$805,393	$89,488	$715,905	9/18/2019	8/31/2022	No	No	No
9JK08	University of Miami	Kunjan Dave, PhD	$805,409	$67,116	$738,293	9/4/2019	9/30/2022	No	No	No
9JK09	University of Miami	Sulagna Banerjee, PhD	$805,409	$67,116	$738,293	9/24/2019	9/30/2022	No	No	No
9JK10	University of South Florida	Rex Philpot, PhD	$771,341	$235,689	$535,652	8/14/2019	7/31/2022	No	No	No

1. **Grant #:** 9JK01 Nutritional Interventions to Alleviate Cardiovascular Disease Mediated by Tobacco Use

   Principal Investigator: Gloria Salazar, PhD

   Organization: Florida State University

   Grant Progress Report: Smoking and aging are two major risk factors in cancer and CardioVascular Disease (CVD) development. Although recent reports showed that smoking stimulates senescence (aging) in the lung, it is unknown whether smoking also accelerates senescence of the cardiovascular system. In this proposal, we proposed the novel hypothesis that cigarette smoke and nicotine accelerate vascular senescence promoting the development of atherosclerosis. We hypothesize that aging and smoking activate a common molecular mechanism that depends in part on the NADPH oxidase Nox1 (an enzyme that produces reactive oxygen species) and activation of the senescence associated secretory phenotype (SASP), a process by which senescent cells modify the microenvironment inducing inflammation, oxidative stress and tissue dysfunction. We demonstrated that polyphenols isolated from blackberries reduce oxidative stress and senescence induced by angiotensin II (Ang II), a strong stimulator of senescence and CVD, by inhibiting Nox1 in vascular smooth muscle cells (VSMCs). Further, overexpression of Nox1 and nicotine treatment induce senescence. Our novel preliminary data show that blackberry supplementation reduced senescence and atherosclerosis in ApoliPOprotein E (ApoE) knock out mice in vivo and that nicotine alone is enough to increase atherosclerosis in the ApoE knock out mice. Nox1 produces superoxide and has a dual role in CVD and cancer. In the cardiovascular system, Nox1 activation by Ang II promotes atherosclerosis and hypertension, while in the lung Nox1 promotes metastasis of lung cancer cells. This proposal will test the hypothesis that inhibition of Nox1 by blackberry polyphenols reduces the senescence-associated secretory phenotype (SASP), thus diminishing senescence and atherosclerosis caused by tobacco smoke. This hypothesis will be tested through the following aims: determine the contribution of Nox1 to the development of the SASP and senescence of VSMCs induced by cigarette smoke and nicotine; 2) define the molecular mechanism by which blackberry polyphenols regulate the Nox1/SASP pathway to reduce senescence of VSMCs; and 3) determine the role of blackberry polyphenols in vascular senescence and atherosclerosis induced by cigarette smoke and nicotine in vivo. In this reporting period significant advances were made in aims 1 and 3 considering the restrictions in research activities due to COVID-19. In Aim 1, optimal conditions to treat vascular smooth muscle cell (VSMC) with nicotine were established to determine the expression of SASP components. Among these components, the research team found that the metalloproteinases (MMPs) MMP2 and MMP3 are upregulated by nicotine. MMPs are enzyme that degrade the extracellular matrix, such as collagen, which allows smooth muscle cells to migrate into the lumen of arteries to initiate the formation of the atherosclerotic plaque. The activity of MMP2 was also increased, which was measured using zymography. For Aim 3, mice exposed to cigarette smoke or nicotine in drinking water for four months showed a significant increase in atherosclerosis and senescence in the aorta. Overall, these data support the proposed hypothesis and demonstrate the negative effects of smoking and nicotine in the cardiovascular system. These results will help to educate Floridians of the consequences of nicotine products as inducers of cardiovascular aging.

Follow-on Funding: National Institute of Health (NIH) R01, Gloria Salazar

Collaborations: None at the time of reporting

Journals: Centner AM, Bhide PG and Salazar G. Nicotine in senescence and atherosclerosis. Cells 2020 April 22;9: E1035. doi: 10.3390/cells9041035

Cullen AE, Centner AM, Deitado R, Fernandez J and Salazar G. (2020). The impact of dietary supplementation of whole foods and polyphenols on atherosclerosis. Nutrients 2020, 12(7): E2069, doi: 10.3390/nu12072069

Patents: None at the time of reporting

2. Grant #: 9JK02 Early Life Exposures and Risk of Developing Ovarian Cancer

Principal Investigator: Shelley S. Tworoger, PhD

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

Grant Progress Report: Ovarian cancer is the most fatal gynecologic malignancy in Florida and is responsible for nearly 1000 deaths annually statewide. With respect to other U.S. states, Florida ranks number two for new ovarian cancer diagnoses and ovarian cancer specific deaths according to American Cancer Society estimates for 2020. With no effective screening tests available, identifying new risk factors is crucial to reduce the burden of this deadly disease. Several lines of evidence suggest that childhood and adolescence are critical periods when exposures can alter how the ovaries develop and impact the likelihood of future ovarian cancer development. During the current reporting period, the research staff conducted analyses of early cigarette smoking exposure and early life abuse in relation to risk of ovarian cancer using data from two large studies of U.S. women that have been followed by biennial questionnaires since 1976. In analyses examining the association between early life smoking exposure and risk of ovarian cancer, age when started smoking and having a mother who smoked while pregnant were not related to subsequent risk of ovarian cancer. However, women with a parent who smoked in the home during their childhood had an 18% higher risk of ovarian cancer, especially non-serious or low-grade serous tumors, compared with women who were not exposed to smoking in the home. The association between early life exposure to smoking in the home and risk of ovarian cancer was similar among women who became smokers themselves as adults and those who never smoked themselves. These results suggest that exposure to toxins in cigarette smoke in early life while the ovaries are developing may contribute to later development of ovarian cancer.

In analyses of early life physical, emotional, and sexual abuse and risk of ovarian cancer, the research staff observed suggestive associations of greater ovarian cancer risk among women who experienced higher levels of physical and emotional abuse and sexual abuse. However, after accounting for symptoms of depression and Post-Traumatic Stress Disorder (PTSD) in adulthood, all of the suggestive associations substantially attenuated. In prior research, an over 2-fold increased risk of ovarian cancer was observed among women with high PTSD symptoms versus women who did not experience trauma. Additionally, prior studies have demonstrated an association of trauma and stress with diminished immune function and increased inflammation, two processes known to contribute to ovarian cancer development. Together, these results

suggest that abuse may impact ovarian carcinogenesis primarily through its role as a driver of post-traumatic stress. Manuscripts describing these results are in preparation. Disseminating these results will contribute to justification for targeted messages to parents to avoid smoking inside the home as well as initiatives to prevent abuse, as both actions may help decrease incidence of ovarian cancer. In addition, these results may help researchers improve prediction models for ovarian cancer, which would have direct implications for health care delivery and be a substantial benefit to Floridians given the significant burden of ovarian cancer in Florida.

Follow-on Funding: None at the time of reporting

Collaborations: University of Florida: Co-investigator Danielle Jake-Schoffman, PhD is an Assistant Professor in the Department of Health Education and Behavior in the College of Health and Human Performance at the University of Florida, Gainesville, Florida. There are currently no University of Florida students performing research under this research project.

Journals: None at the time of reporting

Patents: None at the time of reporting

3. **Grant #:** 9JK03 Immunotherapy in Combination with PARP Inhibition in Advanced Cervical Cancer Patients Functionally Competent or Deficient for the Fanconi Anemia Repair Pathway

Principal Investigator: John P. Diaz, MD

Organization: Miami Cancer Institute, Baptist Health South Florida

Grant Progress Report: Cervical cancer is the third most common gynecological cancer in the United States. Women who smoke and are Human Papilloma Virus (HPV)-positive have up to three times the risk of developing cervical tumors compared to non-smokers. The incidence remains elevated in the Hispanic population. Disparities in this ethnic subgroup correlate with poor access to healthcare and lower socioeconomic status. Hispanics comprise the largest ethnic minority in Florida; therefore, the disease is a public healthcare concern in the state. A newly approved option in second-line treatment for advanced cervical cancer is the immune checkpoint agent pembrolizumab, an IgG4 monoclonal antibody which blocks binding of Program Death (PD1) to Program Death Ligand (PDL1) and PDL2, helping restore T-cell immune response. This was based on a low Overall Response Rate (ORR) of only 13.3%. To increase the efficacy of immune checkpoint blockade, a proposed concept is to increase Tumor Mutational Burden (TMB) and neoepitopes expressed on cancer cells. This could be achieved through combining immunotherapy agents with compounds causing DNA damage or inhibition of DNA repair, such as poly (Adenosine DiphosPhate [ADP]–ribose) polymerase inhibitors, which lead to accumulation of DNA single-strand and consequently double-strand breaks in patients with BRCA mutated tumors who are innately deficient in Homologous Repair (HR). BRCA genes collaborate with several others in the Fanconi Anemia (FA) HR pathway, so we developed an immunofluorescence based method, FancD2/DAPI/Ki67 (FA Triple Stain Immunofluorescence FATSI), which permits the observation of FancD2 foci formation (or lack thereof) in the nucleus of proliferating cells in paraffin-embedded tumor tissues. Staff screened over 600 patients in a clinical trial and found a functional deficiency in 29% of solid tumors. Staff also showed it is safe to administer the poly-ADP ribose polymerase (PARP) inhibitor veliparib, combined with the DNA

damaging agent mitomycin C, to patients with FA deficient tumors. Staff are completing a trial of pembrolizumab in solid tumors functionally competent or deficient for the FA repair pathway with encouraging results in two cervical cancer patients with Hispanic ethnicity. Therefore, the team hypothesize that cervical cancer patients will have improved responses to pembrolizumab when given in combination with olaparib, a potent PARP inhibitor, and the FATSI assay could serve as an indicator of tumor response to immune checkpoint inhibition in FA deficient tumors. To support this hypothesis, staff will undertake these research aims: 1) Assess the efficacy of the combination of pembrolizumab and olaparib in patients with advanced cervical cancer; 2) Investigate whether functional deficiencies in the FA pathway of cervical cancers correlate with improved response to the combination. To accomplish Aim 1, the team will perform a Phase II efficacy study of the combination in advanced cervical cancer patients after failure of first-line standard therapy at the Miami Cancer Institute and other Florida satellites. The primary objective is immune ORR to the combination. The study will accrue a total of 44 patients in a Simon two-stage design for goal efficacy of >35%. To accomplish Aim 2, the team will perform the FATSI assay, next generation sequencing and TMB of tissue and blood specimens of patients at baseline and correlate with response to therapy.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

4. **Grant #:** 9JK04 Adoptive PM21-NK Cells with PD-L1 Blockade for Treatment of Lung Cancer

   Principal Investigator: Alicja J. Copik, PhD

   Organization: University of Central Florida

   Grant Progress Report: Non-small cell lung carcinoma makes up 85% of all lung cancer cases and is the leading cause of cancer-related death. Although immunotherapy with checkpoint inhibitors has been a breakthrough for patients with advanced stage lung cancer, the response rate is still low, and many patients eventually relapse. This project aims to develop clinically translatable immunotherapeutic strategies for lung cancer treatment to increase the response rate to the approved checkpoint inhibitor therapies and to lower relapse rate. To achieve the proposed goals, the project is leveraging the unique capabilities of Natural Killer (NK) cells multiplied to great numbers in our laboratory and reprogrammed to be highly activated through exposure to cellular membrane particles (PM21) or exosomes (EX21) derived from IL21 expressing feeder cells (K562mbIL2141 bbl, mb21FCs). These PM21-particle stimulated NK cells produce IFN-gamma in response to encounters with tumor cells and make them induce PD-L1. Tumors that now have Program Death Ligand (PD-L1) on their surface can be targeted by therapeutic humanized antibody against PD-L1 and further enhance tumor killing by NK cells via Antibody-Dependent Cell Cytotoxicity (ADCC). Killing via ADCC is more resistant to immunosuppression and represents the most powerful mode of NK cells cytotoxicity. NK cells are also known to recruit other components of the immune system, such as dendritic cells, as well as cytotoxic and helper T cells to further direct

complete elimination of cancer. The team hypothesize that this approach has the potential to tum "cold tumors", "hot" to greatly improve treatment outcomes. Staff's method using nanoparticles (PM21) and exosomes (EX21) derived from mb21FCs further introduces new therapeutic dimensions by 1) a feeder cell-free NK cell propagation and stimulation system that can produce high numbers of therapeutic NK cell; 2) persistence of response through repeat injections of activated NK cells; and 3) reprogramming of NK cells ex vivo or in vivo, without genetic modification of the immune cells. Specific Aims are testing parameters to inhibit the immunosuppressive environment and enhance NK cell anti-tumor activity. The treatment of recurrent or relapsed cancer is a difficult challenge for traditional cancer therapies, but recent results from Phase 1 trials in Acute Myeloid Leukemia (AML) using activated NK cells show a 30% decrease in relapse over historical data. Collectively, new alterations to NK cell-based therapeutics are expected to advance treatment responses in order to improve outcomes of patients with advanced stage lung cancer that did not respond to or relapsed on antiPD1/PDL1 therapy.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

5. **Grant #:** 9JK05 Clinically Efficient Strategies to Address Tobacco Smoke Exposure in Pediatric Practice

   Principal Investigator: Ramzi G. Salloum, PhD

   Organization: University of Florida

   Grant Progress Report: The study protocol was approved by the University of Florida Institutional Review Board (IRB) on March 17, 2020 (Protocol No. IRB201901785). In preparation for the planned trial, research assistants were deployed at participating University of Florida Health clinics to assist patients with MyChart activation since December 2019. Primary delivery of the intervention in the trial will occur via MyChart. The status of MyChart activations is as follows: At the Main Street Family Medicine Clinic, portal activation has increased from 12% (November 2019) to 32% (June 2020) among patients 12-17 years old, and from 15% to 23% among patients 0-11 years old. At CMS Schiebler, portal activation has increased from 36% (November 2019) to 39% (June 2020) among patients 12-17 years old, and from 26% to 37% among patients 0-11 years old. At Magnolia Parke, portal activation has increased from 24% (January 2020) to 37% (June 2020) among patients 12-17 years old, and from 42% (January 2020) to 56% (June 2020) among patients 0-11 years old. At Tower Square, portal activation has increased from 26% (January 2020) to 32% (June 2020) among patients 12-17 years old, and from 34% (January 2020) to 45% (June 2020). At Tioga, portal activation has increased from 40% (January 2020) to 43% (June 2020) among patients 12-17 years old, and from 51% (January 2020) to 58% (June 2020) among patients 0-11 years old. Research assistants have not been able to continue their operations in all clinics since April due to the COVID-19 pandemic. However, staff are currently working with the Privacy and Risk Management offices at the University of Florida on exploring options for possible remote

MyChart recruitment and activation.

In addition, staff continued to work closely with the Medical Directors at the participating clinics as well as with the MyChart programming team on further development of the Bright Futures Questionnaires in MyChart. The following questionnaires have now moved into production: 1-month old visit, 2-month old visit, 4-month old visit, 6-month old visit, 9-month old visit, 12-month old visit, 15-month old visit, 18-month old visit, 2-year old visit, 4-year old visit, 11-14 year old visit, and 15-17 year old visit.

Follow-on Funding: Florida Department of Health; Clinically Efficient Strategies to Address Tobacco Smoke Exposure in Pediatric; Ramzi G. Salloum, PhD; $ 404,909.00

Collaborations: The current project is to be conducted entirely at the University of Florida and there are currently no relevant collaborations to report with other postsecondary educational institutions. At the University of Florida, there are currently two students involved in the project, one doctoral student from the Department of Health Outcomes and Biomedical Informatics and one medical student who is involved through the University of Florida Medical Student Research Program.

Journals: None at the time of reporting

Patents: None at the time of reporting

6. **Grant #:** 9JK06 Testing Novel Drug Combination for Pancreatic Cancer

Principal Investigator: Maria Zajac-Kaye, PhD

Organization: University of Florida

Grant Progress Report: Pancreatic Ductal AdenoCarcinoma (PDAC) is the third leading cause of cancer related deaths in the United States and is projected to become the second leading cause of deaths by 2030. The burden of PDAC is high in Florida where more than 3,000 individuals lost their lives last year. Surgery provides the only curative therapy for PDAC but less than 20% of patients are suitable candidates due to challenges to detect cancer when it is surgically removable. While modest improvements in survival have resulted from the use of complex and toxic chemotherapy regimens such as FOLFIRINOX in patients with advanced disease, survival remains largely unchanged. This realization led to the Recalcitrant Cancer Research Act H.R.733 passed by Congress which focused on PDAC and emphasized the broad public interest in testing new treatment approaches. Therefore, to reduce PDAC burden in Florida, staff study the role of novel targeted therapies to reduce PDAC burden amongst a diverse population of PDAC patients.

This year´s goal was to use genetically engineered mice that conditionally express mutant KRAS and human Thymidylate Synthase (TS) in the pancreas to test novel drug compounds for treatment of pancreatic cancer. TS is an essential enzyme for DNA synthesis and repair aberrantly overexpressed in a range of human cancers including PDAC. The team demonstrated that overexpression of TS in the pancreas promoted aggressive PDAC development and markedly reduced survival of KRAS mutant mice. Thus, the goal of this proposal is to develop new treatments for pancreatic cancer using unique TS inhibitors identified in the team's laboratory. Since the preclinical data shows that TS inhibitors synergistically

enhance RAS/PI3K/AKT/mTOR inhibition in vitro, the team proposed in this project to test new TS inhibitors alone or in combination with mTOR inhibitors using the novel hTS/Kras PDAC model. In the past year staff determined that new TS inhibitor designated compound P is not toxic in mice and established the maximum tolerated dose for compound P when combined with Everolimus in vivo. The team also confirmed the antitumoral effect of the drug combination in vivo, and initiated treatment of hTS/Kras genetically engineered mouse model. It was determined that animals treated with drug combination do not present any signs of toxicity or weight loss. The plan is to expand the number of animals per group in the next months to determine if compound P alone or in combination will prolong survival. In addition, staff tested the effect of TAK-228 (potent and highly selective mTORC1/TORC2 inhibitor that is being tested in several clinical trials for different malignancies) and emsirolimus (another rapalog as Everolimus). Staff established the concentration of both drugs alone that will be used in future synergy studies in vitro. Findings from this translational study will lay the groundwork for a personalized investigator-initiated clinical trial at the University of Florida that will reduce PDAC mortality in the Floridian population. Better understanding of this targeted drug combination will enable to treat PDAC patients, improve quality of life and clinical outcomes.

Follow-on Funding: None at the time of reporting

Collaborations: Due to the COVID-19 pandemic, the undergraduate students that were receiving training and performing research under this project were not allowed by the University of Florida to be in the laboratories, so they could not be involved during this quarter of this project. The team conducted several meetings with Dr. Trevino, who is a collaborator in this study, and also organized tumor samples for PDX studies that will be initiated for Aim 2 in year two of the grant.

Journals: None at the time of reporting

Patents: There is a patent approved that is not the outcome of the project but is part of overall research from the group related to the project.

U.S. Patent Application No.:15/737,545; Title: COMPOSITIONS FOR THE TREATMENT OF CANCER AND USES THEREOF; Filing Date: December 18, 2017; Inventors: Maria Zajac-Kaye and Rony Francois. UF Ref. No.: T15748 Approval date 07/06/2020; Filing institution: University of Florida.

7. Grant #: 9JK07 Mechanism of Smoking Induced Promotion of Pancreatic Cancer

Principal Investigator: Dr. Vikas Dudeja, MD

Organization: University of Miami Miller School of Medicine

Grant Progress Report: Pancreatic cancer is one of the few cancers for which survival has not improved substantially over nearly 40 years. Poor understanding of its pathogenesis is one of the major reasons for lack of progress. Due to its unique demographics, including aging population and a large proportion of immigrants, Floridians unfortunately bear more than a fair share of deaths due to pancreatic cancer.

While the pathogenesis of pancreatic cancer is far from clear, some risk factors make a person more likely to get the disease. For instance, according to the American Cancer Society, the risk

of developing pancreatic cancer is about twice as high among smokers compared to those who have never smoked. While the association of smoking and pancreatic cancer is clear, our understanding of how smoking leads to pancreatic cancer, and for that matter any cancer is still evolving. In the laboratory of the Principal Investigator (PI) (Dr. Dudeja), recent research has made a stark observation that gut microbiome promotes pancreatic cancer. Furthermore, new preliminary data also demonstrated that smoking modulates gut microbiome and that smoking induced promotion of pancreatic cancer requires participation of gut microbiome. These are very significant findings as elucidation of the mechanism by which smoking promotes pancreatic cancer initiation and progression will lead to development of novel therapeutic strategies for this formidable disease. Based on the preliminary data from PI's laboratory and literature, the PI had put a novel hypothesis that "Smoking modulates gut microbiome to a pro-tumorigenic composition, which in turn promotes pancreatic cancer by modulating anti-cancer immune response." The current grant is poised to evaluate this hypothesis through following specific aims.

Specific Aim 1: Evaluation of the role of changes in gut microbiome in smoking induced progression of pancreatic cancer.
Specific Aim 2: Elucidation of the smoking induced gut microbiome changes.
Specific Aim 3: Elucidation of the role of gut microbiome mediated immune modulation in smoking induced promotion of pancreatic cancer.

Since the administration of this award (~9 months ago), despite difficulties in the conduct of research imposed by the raging COVID-19 pandemic significant progress has been made. In Aim 1, staff have used two strategies to simulate smoking: Use of nicotine-derived nitrosamine ketone (NNK), one of the carcinogens found in cigarette smoke; and use of smoking machine, which exposes the experimental animals to smoking. Using the NNK model as well as the smoking machine, staff have demonstrated that exposure to smoking promotes pancreatic, colon and bladder cancer and this effect of smoking on tumor growth can be mitigated by depletion of gut microbiome. These data strongly support our hypothesis that gut microbiome changes mediate the effects of smoking in not only pancreatic, but many other cancers. Thus, the implication of the current research is broad and far-reaching.

Smoking promotes pancreatic, colon and bladder cancer in a gut microbiome dependent fashion. NNK leads to increase tumor growth of subcutaneous tumors of KPC pancreatic cancer cell line. However, NNK is unable to increase tumor growth in the absence of gut microbiome (NNK + ABX group). Gut microbiome was depleted by a cocktail of poorly absorbable antibiotics including ampicillin, neomycin, metronidazole and vancomycin. The same effect was seen in another pancreatic cancer cell line panc02. (C-E) Smoke exposure leads to increase tumor growth of pancreatic, bladder and colon cancer cell lines tested. However, smoking is unable to increase tumor growth in the absence of gut microbiome (Smoke + ABX group). This highlights that gut microbiome is essential for smoke to produce its tumor permissive effects. The team's efforts over the next years of this grant will be focused on confirming these findings in genetically engineered mouse models and models of metastatic pancreatic cancer and in models of minimal residual disease. This is important to fully understand the implications of the findings.

Aim 2 of the current grant is focused on elucidating the effect of smoking on the gut microbiome. For this, staff have performed 16s rRNA gene analysis on the stool of NNK and smoke exposed mice to elucidate smoke induced gut microbiome changes. The stool of NNK and smoke exposed mice is significantly different from control mice highlighting that NNK/smoke exposure alters the gut microbiome. Efforts over the next years of this grant will be focused on elucidating

the specific bacterial communities which mediate these effects. This will lead to devising strategies to modulate gut microbiome to counteract harmful effects of smoking.

The Aim 3 of the current grant is focused toward understanding the mechanism how smoking induces changes in gut microbiome modulate the tumor growth. Staff have specifically looked at the effect of smoking on the anti-cancer immune response. For this, staff have done flow cytometric analysis on tumors of mice exposed to NNK with or without gut microbiome depletion to evaluate the role of gut microbiome mediated immune changes. NNK exposure decreased antitumor IFN-γ +ve cytotoxic CD8 T cells.

Interestingly, in the absence of gut microbiome there were significantly increased IFN-γ +ve cytotoxic CD8 T-cells even in NNK exposed mice. Similarly, smoke exposure decreased infiltration of cytotoxic CD8 T-cells and promoted tumor promoting Myeloid Derived Suppressor Cells (MDSCs) infiltration. Interestingly, in the absence of gut microbiome there were significantly increased cytotoxic CD8 T-cells and decreased MDSCs infiltration even in smoke exposed mice, highlighting that gut microbiome interacts with the immune system to mediate immunosuppressive effects of smoke. In summary, our data suggest that the gut microbiome is essential for the tumor promoting effects of cigarette smoke/NNK and targeting this dysbiosis could emerge as a novel strategy to mitigate the worse outcomes of pancreatic cancer in smokers. Staff plan to use humanized gut microbiome avatar mice in the future to test our hypothesis in the setting of human gut microbiome.

Follow-on Funding: None at the time of reporting

Collaborations: Currently collaborating with Baylor College of Medicine to perform metabolomics on the samples generated from this project

Journals: None at the time of reporting

Patents: None at the time of reporting

8. Grant #: 9JK08 Nicotine Exposure and Intracerebral Hemorrhage

Principal Investigator: Kunjan R. Dave, PhD

Organization: University of Miami

Grant Progress Report Smoking is one of the main risk factors for Spontaneous IntraCerebral Hemorrhage (sICH): the deadliest subtype of stroke. Despite being the cause of significant morbidity and mortality, sICH remains the least treatable stroke subtype. Continued cerebral bleeding leading to hematoma expansion is highest in the first three hours after symptom onset and may continue in a large number of patients between three and 24 hours after onset. Hematoma volume in sICH patients correlates with the 30-day mortality rate. Currently, there is no proven therapy to prevent hematoma expansion in sICH patients, and thus clinicians are unable to offer more than supportive care. Several epidemiological studies demonstrated the deleterious effects of smoking/tobacco use in sICH patients. These effects include increased risk of sICH, larger hematoma expansion, and poor post-sICH outcomes. Despite several clinical studies indicating the deleterious effects of smoking/tobacco use in sICH patients, the field is lacking confirmatory systematic preclinical studies evaluating the effects of smoking on outcomes following sICH. The main goal of the proposal is to achieve

the goals of the James and Esther King Biomedical Research Program by improving the health of Floridians. In this project, staff proposed to test the hypothesis that chronic nicotine exposure will worsen outcomes following sICH and red blood cell microparticles (RMP: hemostatic agent) will be able to limit hematoma growth in a clinically relevant animal model of sICH. Staff propose to test this hypothesis by determining the effect of chronic nicotine exposure on outcomes following sICH, the mechanisms by which chronic nicotine exposure increases hematoma volume post-sICH, and if RMP treatment improves post-sICH outcomes in chronic nicotine-treated rats via limiting hematoma growth under the last translational aim. In the first three quarters of the project, staff made the following accomplishments. Confirmed that chronic nicotine treatment in young male rats resulted in larger hematoma volumes and worsened behavioral outcomes following collagenase-induced sICH. Started experiments to evaluate if chronic nicotine treatment in young female rats also results in worse outcomes following collagenase-induced sICH. Study targeted to evaluate levels of biomarkers of blood brain barrier (BBB: a protective barrier between blood and brain) indicate potentially weakened BBB in chronically nicotine-treated young male and female rats. As a functional assay to evaluate permeability of BBB, staff performed experiments to evaluate permeability of a commonly used tracer (evans blue dye) in chronically nicotine-treated young male and female rats. These studies indicate increased leakage/permeability of Blood Brain Barrier (BBB) in nicotine-treated group. Studies so far indicate that chronic nicotine treatment results in increased hematoma growth (more bleeding) in young male rats potentially via compromised BBB permeability.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

9. Grant #: 9JK09 Role of Microenvironment in Enrichment of Aggressive CD133 Population in Pancreatic Cancer

Principal Investigator: Sulagna Banerjee, PhD

Organization: University of Miami - Miller School of Medicine

Grant Progress Report: Tobacco smoking is considered to be one of the major risk factors for pancreatic cancer, a disease with very poor survival rates. Recent research has shown that tobacco and the carcinogens like NNK present in it can promote pancreatic tumorigenesis and significantly alters the tumor microenvironment and pushes the tumor towards a poorer prognosis. Research has shown that the components of tobacco drive the KRAS mutation (a commonly occurring mutation in Pancreatic ductal adenocarcinoma PDAC) along with inadvertently selecting for a population that is enriched for a population of cells that have increased survival advantage. Epidemiological studies using large cohorts of patients have shown that usage of tobacco decreases the survival in PDAC patients. Further, nicotine one of the major components of cigarette smoke also activates the HGF/Met activating pathway in the tumor, that promotes a microenvironment dependent signaling in the pancreatic tumor, thereby promoting metastasis. The funded grant is geared towards understanding how the

fibro-inflammatory microenvironment in pancreatic cancer can select for a population of CD133+ cells that contributes to the aggressive biology of the tumor; and whether this component of the pancreatic tumor microenvironment can be targeted in order to improve the current dismal prognosis in this disease. In the last year, research staff has established that the inflammatory cytokine IL6 produced by the stromal cells can enrich for CD133+ cells in the tumor. Since aggressive cells have high expression of metastatic genes and pro-survival genes, staff evaluated the expression of these genes after treating pancreatic cancer cells (MIA-PACA2) with conditioned media from stellate cells. Treatment with conditioned media from pancreatic stellate cells upregulated the expression of pro-survival genes in the pancreatic cancer cells. Studies further showed that treatment of pancreatic cancer cells with conditioned media from stellate cells increased invasiveness as well as expression of metastatic genes in pancreatic cancer. Research staff also confirmed preliminary observations that stromal cells enriched for CD133+ cells in pancreatic cancer using cell line Su86.86. Studies showed that when treated with conditioned media from pancreatic stellate cells, Su86.86 also enriched for CD133+ cells. Additionally, co-culture with stromal cells also enriched for CD133+ population in pancreatic cancer. Preliminary data showed that presence of stroma increased tumor initiation. Staff have now calculated the tumor initiation frequency in the animals with stromal cells and without stromal cells. Results show that in the presence of stromal cells the tumor initiation frequency of pancreatic cancer cells is much higher compared to those without stromal cells. Further, in vivo studies also show that progression was higher in tumors that were co-implanted with stromal cells compared to those that did not have stromal cells. Preliminary studies had indicated that stroma secreted IL6 can promote “stemness” in pancreatic cancer. Since one of the features of cancer stem cells is chemoresistance, staff treated pretreated pancreatic cancer cells BxPC3 and CFPAC with IL6 prior to their treatment with Gemcitabine. Studies showed that IL6 decreased apoptotic cells (studied by Annexin V staining) in the presence of gemcitabine indicating induction of chemoresistance. Unfortunately, at this point (March 16, 2020) labs were shut down due to the Covid#19 pandemic. Experiments could not be conducted during this period. The metabolic and immunological studies will be done once the labs are fully operational.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

10. Grant #: 9JK10 The Effects of Chemotherapy for Breast Cancer on the Central Nervous System

Principal Investigator: Rex M. Philpot, PhD

Organization: University of South Florida

Grant Progress Report: Although not normally a part of an update of this type, it should be noted that 4.5 months of effort were undermined by uncontrollable circumstances. Work on this project could not begin until 8/23/19, when the fully executed award was received from the Florida Department of Health. Further, due to the COVID-19 crisis, the University was shut down from 3/14/20 to 6/1/20, to all but essential personnel. During this time only the most basic research efforts could be made, with the primary focus on maintaining the mouse model colony

and on treating those animals that developed cancer. Nevertheless, excellent progress was made during fiscal year one, with the foundation for all aspects of this project firmly established and ready to build upon in year two. The most critical research successes from year one is outlined below:

The team has successfully established a colony of mice that reliably develop breast cancer approximately 120 days after birth. Using this model, staff have been able to demonstrate that two drugs used to treat breast cancer under study (Cyclophosphamide and Doxorubicin) can interfere with learning and memory in mice with cancer. We have also demonstrated that cancer is not necessary for the drugs to cause problems with learning and memory, but that any issues with learning and memory may be worse when cancer is present. Thus, staff have developed an effective animal model of chemo-brain for these studies.

The team has determined that the experimental drugs being tested (Xanomeline Oxylate and VU 0357017) to prevent these problems with learning and memory do not cause the tumors to grow faster or cause an increase in the number of tumors. Importantly, these experimental drugs do not interfere with the effectiveness of the cancer treatment drugs, cyclophosphamide and doxorubicin. Therefore, Xanomeline Oxylate and VU 0357017 are safe to use during cancer treatment for the prevention of chemo-brain.

Measurements from blood samples collected from mice with and without tumors indicate that the two drugs used to treat breast cancer not only significantly reduces circulating estradiol, as predicted, but that it also reduces circulating progesterone, which was unexpected. Since the progesterone concentrations affect the number of neurons in the brain that produce chemicals involved in learning and memory, this is a significant finding that requires further exploration in relation to the hypothesis.

The team has collected several brains from these animals following treatment and the measurement of learning and memory capacity. These brains will be examined for changes in the number of neurons that produce chemicals involved in learning and memory and to determine if the experimental drugs being tested protect the brain from loss of these types of neurons. However, these measurements take considerable time and cannot be started until all the mice have been treated and tested for learning and memory deficits.

Follow-on Funding: None at the time of reporting

Collaborations: University of South Florida, Morsani College of Medicine Department of Pharmacology and Physiology Robert Bothello, Graduate Student

Journals: None at the time of reporting

Patents: None at the time of reporting

James and Esther King Biomedical Research Program
Appendix M
Fiscal Year 2019-2020 Active Grants
Funding Year 2017-2018

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
8JK01	Florida Atlantic University	Gregg Fields, PhD	$708,044	$478,408.00	$459,272.00	5/11/2018	4/30/2021	No	Yes	Yes
8JK02	H. Lee Moffitt Cancer Center	Jennifer Permuth, PhD	$1,360,857	$1,020,636.00	$340,221.00	5/03/2018	3/31/2021	No	No	No
8JK03	H. Lee Moffitt Cancer Center	Nagi Kumar, PhD	$708,044	$315,079.80	$392,964.20	5/03/2018	3/31/2023	No	No	No
8JK04	University of Florida	Frederic J. Kaye, MD	$1,360,857	$586,124.76	$774,732.24	6/06/2018	3/31/2023	No	No	No
8JK05	University of Florida	Sergei Kusmartsev PhD	$816,514	$612,387.00	$544,342.00	5/04/2018	3/31/2021	Yes	Yes	No
8JK06	University of Florida	Linda F. Hayward, PhD	$816,514	$544,344.00	$272,170.00	5/07/2018	3/31/2021	No	No	No
8JK07	University of Miami	Sabita Roy, PhD	$816,514	$612,387.00	$204,127.00	4/25/2018	3/31/2021	No	No	No
8JK09	University of South Florida	Tomar Ghansah, PhD	$816,514	$589,707.00	$226,807.00	4/17/2018	3/31/2021	No	Yes	Yes

1. **Grant #:** 8JK01 Inhibition of Tumor Cell Surface Proteolysis

   Principal Investigator: Gregg B. Fields, PhD

   Organization: Florida Atlantic University

   Grant Progress Report: Matrix MetalloProteinase 14 (MMP-14)/MT1-MMP is a type I transmembrane cell-surface protease overexpressed in many tumors. The increased presence of MT1-MMP is associated with poor prognosis in patients with melanoma, small cell lung cancer, tongue squamous cell carcinoma, head and neck carcinoma, bladder cancer, and breast cancer, amongst others. Increased tumor cell production of MT1-MMP enhances tumor growth, invasion, and metastasis. Overall, the production of MT1-MMP correlates to poor prognosis in a number of tobacco-related cancers and the collagen-cleaving ability of MT1-MMP is critical to the progression of a number of tobacco-related cancers. A mechanistic examination of MT1-MMP at the cell surface would unravel the influences of cell surface binding partners on MT1-MMP activities and set the stage for the development of unique MT1-MMP inhibitors. The present project utilizes cutting-edge technologies to examine, on a molecular level, how a cell surface protease (MT1-MMP) functions in its native environment. In addition, the cell surface nature of MT1-MMP will be utilized to design novel inhibitors. The specific aims to achieve these goals are as follows: quantitative analysis of MT1-MMP activity on the cell surface, including the modulation of activity by specific MT1-MMP domains and binding partners; and development of inhibitors of MT1-MMP function based on one-bead-one-compound conformationally constrained libraries targeting secondary binding sites (exosites) within the enzyme. The present work will lead to a detailed, mechanistic understanding of cell surface proteolysis and the exploration of cell surface proteolysis inhibitors based on unique modes of action. Inhibitors will be characterized using three-dimensional invasion models of melanoma.

   In the last year, the team has made progress in three areas. First, the expressional of 14 enzymes, full-length MT1-MMP, soluble MT1-MMP (no transmembrane domain), and full-length MMP-8, with the appropriate tags needed for screening against the combinatorial Peptide-Inspired Conformationally Constrained Oligomer (PICCO) library was completed. The team evaluated the various enzyme constructs to determine which ones had the best activity, so that the most active forms could be used in the library screening. Second, three-dimensional melanoma spheroids were used to quantify MT1-MMP activity, and to quantify inhibitor activity. The three-dimensional system closely mimics the environment that melanoma encounters during the metastatic (cancer spreading) stage. The evaluation of inhibitors in the assay was published. Third, the synthesis of our initial DNA-encoded PICCO libraries was completed. Two libraries totaling 800,000 unique, macrocyclic compounds, consisting of both 2.5-mer and 3.5-mer macrocycles, were screened against His-tagged MT1-MMP and FLAG-tagged MMP-8. Initial results indicating selective binding of a subset of compounds to either MT1-MMP or MMP-8, with one compound identified as a unique MT1-MMP ligand. These compounds provide an important step in the development of therapeutic agents that selectivity inhibit the action of MT1-MMP.

   Follow-on Funding: Florida Department of Health; Memorial Cancer Institute Florida Atlantic University Cancer Center of Excellence; Gregg B. Fields; $Pending

   Collaborations: None at the time of reporting

Journals:
Manikandan Palrasu, Anna M. Knapinska, Juan Diez, Lyndsay Smith, Travis LaVoi, Marc Giulianotti, Richard A. Houghten, Gregg B. Fields, and Dmitriy Minond. A novel probe for spliceosomal proteins that induces autophagy and death of melanoma cells reveals new targets for melanoma drug discovery. *Cell. Physiol. Biochem.* **53**, 656-686 (2019). doi: 10.33594/000000164, PMCID: PMC6990463.

Patents: None at the time of reporting

2. **Grant #:** 8JK02 The Florida Pancreas Collaborative (FPC) Next-Generation Biobank: Reducing Health Disparities and Improving Survival for Pancreatic Cancer

Principal Investigator: Jennifer B. Permuth, PhD

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

Grant Progress Report: Of all tobacco-related cancers in the United States, Pancreatic Cancer (PC) is the deadliest, with a five-year relative survival rate of only 9%. PC just became the third leading cause of cancer deaths and will become the second leading cause around 2020. Florida ranks second in lives lost to PC each year. Striking racial disparities in PC incidence and mortality rates exist nationally and in Florida, with the highest rates among African Americans (AA) followed by Non-Hispanic Whites (NHW) and Hispanic/Latinos (H/L). Reasons for these disparities remain unexplained and underexplored. One factor that contributes to increased morbidity and mortality and diminished Quality of Life (QoL) in most PC patients is cancer cachexia, a metabolic condition characterized by stages of progressive muscle wasting, unintentional weight loss, and fatigue. The goal of our infrastructure grant is to create state resources to conduct basic, clinical, population-based, and translational science that will impact several racial and ethnic groups affected by PC. PC researchers from fifteen Florida cancer centers and hospitals that diagnose and treat a high volume of AA, NHW, and/or H/L individuals with PC have joined forces to: prospectively build a robust 'next-generation biobank' that contains viable tissues, biofluids, medical images, and clinical and laboratory data, all derived from a racial/ethnically diverse cohort of PC patients, and use the biobank to test the hypothesis that cancer cachexia may underlie racial disparities in PC such that AA may present with a higher prevalence of cachexia earlier and more aggressively in the disease process compared to NHW and H/L. We have been productive in building the foundation for this infrastructure project as evidenced by accomplishments in numerous areas including: meeting with scientific and community advisors and co-investigators to discuss and enhance the scope of work; finalizing the study protocol, master consent form, study questionnaires and numerous data collection instruments, and translating pertinent documents into Spanish; obtaining regulatory approval and executing various contracts and agreements; and harmonizing standard operating procedures related to biospecimen and medical image collection, processing, storage, and transfer. We also built a customized platform for data collection, management, and workflow and developed a study logo, recruitment materials, and a study website. Site initiation visits were conducted, and recruitment commenced one site at a time. This infrastructure has potential for great impact because it will address a critical gap in PC research by capitalizing on Florida's large underserved minority PC population and an already established and productive multidisciplinary collaboration with new passionate partners. We will foster a valuable state-wide resource for PC disparities research that will generate impactful findings related to cancer cachexia and enable Florida's researchers to compete for national funding to increase QoL and reduce PC burden, goals in line with the Florida Department of Health and the James and

Esther King Biomedical Research Program.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

3. **Grant #:** 8JK03 Phase II Trial of Investigational Agents to Modulate Intermediate Endpoint Biomarkers, Including Pulmonary Nodules, in Former Smokers

Principal Investigator: Nagi B. Kumar, PhD

Organization: Moffitt Cancer Center

Grant Progress Report: Although the period of report includes 7/1/2019 to 6/30/2020, the clinical trial has been suspended for recruitment from March 2020 to present due to the Covid-19 pandemic. Although screening high risk populations using Low Dose Computed Tomography (LDCT) and smoking cessation programs are critical, former smokers on surveillance are eager to participate in chemoprevention interventions that can further reduce their risk for lung cancer. Our team and others have shown that CURcumin (CUR) and omega 3 Fatty Acids (ω-3 FA) are effective at suppressing Stat3P and NF-κB signaling pathways-that are relevant to lung carcinogenesis-resulting in suppression of proliferation of human lung tumor lines and inflammation responses. More recently, strong evidence has emerged demonstrating the role and mechanism of ω-3 FA as specialized fat mediators, with anti-inflammatory, anti-proliferative and pro-resolving properties towards resolution of cigarette smoke-induced lung inflammation in former smokers. Our team and others have also shown that CUR when combined with ω-3 FA is bioavailable in the lung and produces a more robust antiproliferative effect in lung tumor tissue compared to when these agents administered independently. Based on this evidence, the team hypothesize that a standardized formulation of CUR + ω-3 FA will target molecular pathways that are critical for lung cancers development, leading to a reduction in the overall size and density of nodules, in former smokers. Our hypothesis is that this will be mediated by reducing inflammation and through pro-resolving effects in the nodules. Our hypothesis will be tested by using an experimental design and rigorously evaluating the safety, efficacy and the potential mechanism of a combination of ω-3 FA + CUR or placebo administered for 6 months in former smokers, with lung nodules detected during LDCT screening program. Results of the proposed trial may have significant benefit to former smokers and other high-risk populations in the state of Florida towards lung cancer prevention.

Although the Cancer Center has a large population of potential subjects to be recruited in the trial, this is the most challenging population to recruit into clinical trials. The team has screened over 1450 subjects in this trial. The major reasons for not meeting criteria for inclusion includes the following: lung nodule size and other cancers; current smokers; on medications that make them ineligible; comorbidities; live too far from the cancer center; does not want to quit smoking or has relapsed since CT scan and decline to participate with no reason. Based on these challenges and the recommendations of the Scientific Review Committee, the protocol has been revised to include only high-risk subjects, expanding this group to include those with Lung

RDAS 2 and 3 nodules. The changes have now been approved by all (Scientific Review Committee, IRB and FDA and the State of Florida approved all the changes made and on 6/11/2020. A notice of grant award to continue the trial was granted. There are 12 patients ready to start the trial once the team is permitted to open the trial to recruitment.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

4. **Grant #:** 8JK04 Good Manufacturing Practice (GMP) Production to Allow Phase 1 Clinical Trial Testing Intralesional Delivery of Myxomavirus to Patients with Advanced Small Cell Lung Cancer.

   Principal Investigator: Frederic Kaye, MD

   Organization: University of Florida

   Grant Progress Report: Small Cell Lung Cancer (SCLC) is the most lethal subtype of lung cancer as well as the subtype that is most strongly linked to tobacco exposure. Due to lack of improvement in overall survival over the past decades, Congress specifically emphasized a search for new therapies for SCLC in the Recalcitrant Cancer Research Act H.R.733. In 2019 our team published in a high impact scientific journal our data supporting the delivery of a modified MYXoma Viral agent (MYXV) to stimulate the immune system, to induce tumor specific cell death, and to improve the outcome in SCLC (Oncolytic virotherapy for small-cell lung cancer induces immune infiltration and prolongs survival. J Clin Invest 2019; 129(6):2167-2595). The primary goal of this grant is to translate this pre-clinical data into a novel clinical trial testing the safety and efficacy of MYXV injected directly into lung tumor nodules of patients with advanced SCLC. Over the past 12 months the team has worked i) to develop and optimize the Good Manufacturing Product (GMP) process for MYXV production for the Phase 1 clinical trial, ii) to design animal safety testing, and iii) to complete regulatory requirements for an Investigational New Drug (IND) submission to the Food and Drug Administration (FDA). In the past year, an Interact submission to the FDA and more recently a Pre-IND submission has been completed. While the University of Florida Powell Gene Therapy GMP Viral Production Core laboratory and the Animal Toxicology Core Facility were closed over the past months due to the COVID-19 pandemic, the team continued to meet regularly to plan development of the phase 1 clinical trial. The team has worked to extend our preclinical data on strategies to further enhance host immune response in SCLC and ultimately anticipate testing MYXV intratumoral delivery combined with systemic immune checkpoint inhibitors in patients with advanced SCLC. This work will test an innovative treatment strategy that takes advantage of unique expertise at the University of Florida that has pioneered ultrasound-guided intratumoral drug delivery with expertise in virology, molecular genetics and immunology, and clinical management of patients with lung cancer. The primary goal of this grant is to improve the outcome of patients with advanced SCLC in the State of Florida. This initial James and Esther King Program grant support has also stimulated development of an Inter-Departmental Program within the University of Florida Cancer Center focused on new treatments for SCLC which has resulted in a Federal grant application recently submitted.

Follow-on Funding: None at the time of reporting

Collaborations: This work includes collaborations between basic scientists and clinicians within the State of Florida and has included important collaborations with investigators at the Moffitt Cancer Center who were co-authors on our recent publication listed above. This further supports the goals of the James and Esther King Grant Program to promote collaborations between Cancer Centers within the State of Florida.

Journals: None at the time of reporting

Patents: None at the time of reporting

5. **Grant #:** 8JK05 Dissecting the Mechanisms of Tumor-Induced Tolerance and Immune Suppression in Bladder Cancer

Principal Investigator: Sergei Kusmartsev, PhD

Organization: University of Florida

Grant Progress Report: Smoking tobacco is the most important known risk factor for bladder cancer. Bladder cancer is the ninth most common malignancy worldwide and the fifth most common in developed countries. Approximately 20% of patients are diagnosed with muscle-invasive disease at the time of initial presentation, which will require multiple treatment modalities due to the high rates of disease recurrence, progression and disease specific mortality. Treatment options include chemotherapy, radiation therapy, and radical cystectomy in cases of clinically localized disease and systemic chemotherapy for patients with metastatic disease. Despite this aggressive treatment approach prognosis remains be poor for many patients. The continued poor prognosis observed presents an opportunity for immunotherapy to improve outcomes. However, there is still an unmet need, as the majority of patients do not respond to the immunotherapy in all stages of bladder cancer. A greater understanding of the mechanisms of resistance to immunotherapy will provide alternate strategies to improve bladder cancer care. Purpose of this research project to determine roles of HyAluronan (HA) metabolism in mechanisms of immune evasion and immune tolerance in bladder cancer. Obtained results ultimately provide novel targets for bladder cancer therapy. Several cancer types, including bladder, prostate, brain, lung and breast cancers highly enriched with Hyaluronan (HA). HA accumulation in tumor tissues frequently associated with increased degradation of HA due to deregulated metabolism and increased hyaluronidase (Hyal) expression. Elevated Hyal expression and activity in tumor tissues leads to accumulation of HA fragments with low molecular weight. Our preliminary data strongly suggest an important role for tumor-derived HA and HA-mediate CD44 signaling in tumor-induced immunosuppression. Our observations led us to hypothesize that tumors may evade the immune system by creating protective tolerogenic “shield” in the form of tumor-produced HA, which binds to the CD44-expressing tumor-recruited MDSCs and promoting development of the PD-L1+ macrophages. To test our hypothesis, the following specific aims were developed: Specific Aim1: Determine key molecular components involved in accumulation of immunosuppressive PD-L1-expressing myeloid cells in bladder cancer. Specific Aim 2: Investigate whether targeting HA-CD44 link in bladder cancer could reduce tumor-associated immune suppression and improve anti-tumor immune response in tumor bearing mice and in cancer patients. This research project includes clinical study of patients with diagnosed bladder cancer as well as pre-clinical studies using experimental animal model of bladder cancer. To date, clinical specimens (blood, tumor tissue) from thirty-five

bladder cancer patients have been collected during surgery. Obtained data indicates that metabolism of hyaluronan in bladder tumor tissue is severely affected and characterized by strong fragmentation and increased levels of HA fragments with low molecular weight. Our studies identified the hyaluronidase 2 (Hyal2)-expressing myeloid cells as a novel target for cancer therapy. Based on our findings, a patent was filed with University of Florida and a research article was submitted to the Cancer Research journal for publication (currently under revision for acceptance). Identification of Hyal2-expressing myeloid cells as a novel target opens a window of opportunity for Hyal2 targeting and development of novel cancer immunotherapy approach for cancer treatment.

Follow-on Funding: None at the time of reporting

Collaborations: Established a collaboration with Randall Malcolm VA Medical Center for human bladder cancer research, and a collaboration with College of Veterinary Medicine to study novel biomarkers and develop therapeutic approach for the treatment of bladder cancer in dogs

Journals: *Crispen P. and Kusmartsev S. Mechanisms of Immune Evasion in Bladder Cancer. Cancer Immunology Immunotherapy*. 2020 Jan; 69(1):3-14. **(Published)**

Dominguez Gutierrez P.R, Kwenda E, Donelan W, O' Malley P, Crispen and Kusmartsev S. *Identification of Hyal2-expressing tumor-associated myeloid cells: implication for bladder cancer-related inflammation. Cancer Research, under revision for acceptance* (Manuscript submitted for publication)

Dominguez Gutierrez P.R, Kwenda E, Crispen P. and Kusmartsev S. Development of tolerogenic PD-L1-expressing cell clusters in cancer (Manuscript in Preparation)

Patents:
Patent filed with University of Florida OTL. Invention ID: INV-200073.Identification of Hyal2/Hyal1-expressing tumor-associated myeloid cells: novel target for cancer immunotherapy

6. **Grant #:** 8JK06 The Role of the Gut-Microbiome-Brain Axis in Cardiovascular Disease Following Prenatal Exposure to Nicotine

Principal Investigator: Linda F. Hayward, PhD

Organization: University of Florida

Grant Progress Report: Offspring of smokers have an increased incidence of chronic behavioral problems, cardiovascular disease, and obesity. Although many problems associated with Prenatal Nicotine Exposure (PNE) have been documented, mechanisms underlying these changes remain elusive. Emerging evidence suggests that a common factor behind the many diseases is an imbalance of the bacterial microbes in the gut and associated changes in bidirectional communication between the gut and the central nervous system or the gut-microbiome-brain axis. Thus, the primary goal of this research grant is to evaluate for the first time the impact of PNE on the gut-microbiome-brain axis during two different time points: during pregnancy and later when the offspring are adults. During the second year on the grant (2019-2020), the team completed Aim 1 or the assessment of how PNE alters the maternal gut microbiome and modulates metabolic by-products produced by gut bacteria in the feces and circulation. For Aim 1 three main findings were identified and a manuscript is pending. First,

after 2 weeks of PNE, significant changes in the maternal gut microbiome were identified, including a significant increase in one group of bacteria (phylum Actinobacteria) and a reduction in bacteria in phylum Firmicutes. Second, parallel to changes in the bacterial balance in the gut, there were changes in the types of metabolites, or Short Chain Fatty Acids (SCFAs), found in the feces and circulation. Specifically, PNE induced a general decline in most fecal SCFAs but no change in circulating SCFAs. Third, although PNE did not change maternal body weight, there was a large decline in the circulating leptin, a hormone that signals fat storage. These results suggest the increased incidence of obesity in offspring of smokers may be related to low leptin levels and not a change in SCFAs. In this second year, Aim 2 was completed, which involved evaluating the gut microbiome of the adult PNE offspring. To date, three main findings have been identified. First, adult PNE offspring were heavier and had elevated leptin levels compared to controls. Second, the impact of PNE on the gut microbiome was different in male versus female offspring compared to controls, including an elevation of bacteria in the phylum Verrucomicrobia in females versus phylum Bacteroidetes in males. This was paralleled with declines in only two fecal SCFA levels, that were also sex specific. Third, PNE male offspring were less sensitive to the anorexic effects of the SCFA acetate and this is paralleled by a reduction in brain connectivity/activation, as indicated by MRI. These findings support our original hypothesis and demonstrate that PNE during pregnancy, impacts the gut microbiome and this is linked to differences in offspring gut microbiome and brain related connectivity. Fortunately, the gut microbiome can be inexpensively re-balanced via changes in diet or probiotics. Thus, our research suggests there may be preventative measures that women can take, if they have difficulty quitting smoking during pregnancy, to protect their unborn children from the lifelong consequence of PNE, an important potential benefit for the citizens of Florida.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

7. **Grant #:** 8JK07 Evaluating Mechanisms of Stromal Modulation by Novel Anti-Cancer Drug Minnelide

   Principal Investigator: Sabita Roy, PhD

   Organization: University of Miami - Miller School of Medicine

   Grant Progress Report: Pancreatic cancer is an aggressive malignancy characterized by a dense stroma which makes it recalcitrant to standard chemotherapeutic or surgical treatment strategies. Smoking is one of the factors that contributes to a complex tumor microenvironment in making this disease aggressive. Our proposal examines the efficacy of Minnelide- a pro-drug synthesized in our lab in targeting the tumor stroma cross talk and modulating the tumor microenvironment. Since the last progress report, the team has shown that: Minnelide indeed reprograms the stromal component of the tumor. Minnelide inactivated the CAF cells, which resulted in decreased proliferation well as Vitamin A accumulation. The team investigated the effect of Minnelide on the Extra Cellular Matrix (ECM) components and our results show that Minnelide decreased the levels of fibronection, hyaluronan, collagen as well as MMP2 and MMP9 in the tumor cells as well as in CAFs. Minnelide/triptolide treated decrease in fibronectin

and periostin was rescued by treatment with TGFb. Additionally, following Minnelide treatment, our results show that co-culture of Pancreatic Stellate cells with “inactivated” CAF cells resulted in a decrease in the transcriptional activity of a number of pro-oncogenic pathways in the tumor epithelial cells.

In line with the institutional recommendations, the University of Miami Miller School of Medicine initiated a shutdown of all non- essential research activities since 23rd March 2020. As a result, no progress was made on the project during this period. Additionally, there is an institution wide hiring freeze which will result in further delay in hiring research staff needed for the continuation of the next set of aims for this project.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

8. **Grant #:** 8JK09 SHIP-1: A Potential New Molecular Target for the Treatment of Pancreatic Cancer

   Principal Investigator: Tomar Ghansah, PhD

   Organization: University of South Florida

   Grant Progress Report: Pancreatic Cancer (PC) is a tobacco-related disease that is one of the deadliest cancers. PC inflammatory microenvironment renders current immuno- and chemotherapies ineffective. PC tumor-derived factors cause an expansion of immunosuppressive cells known as Myeloid Derived Suppressor Cells (MDSCs) and regulatory T-cells (Tregs), which suppress anti-tumor immunity. The objective of this study is to develop new therapeutic strategies to reduce MDSC and Treg expansion and improve anti-tumor responses of current PC therapies.

   Our laboratory’s recent work offers a potential solution. Src Homology 2-containing Inositol Phosphatase (SHIP-1) is a vital protein for tumor immunity. The downregulation of SHIP-1 protein levels correlates with the expansion of MDSCs and Tregs in PC mice. We discovered that an anti-inflammatory compound, Apigenin (API) enhances SHIP-1 expression in murine PC model. We hypothesize that PC dampens SHIP- 1 dependent signaling, causing increased MDSC and Treg activity, therefore, creating an inflammatory tumor microenvironment resistant to treatment. However, rescuing SHIP-1 (e.g. API) may reverse this trend, facilitating treatment of PC. The purpose of this research study is to validate that SHIP-1 protein is a potential novel therapeutic target for treating pancreatic cancer (PC). Results from this study will impact the understanding of pancreatic cancer-induced inflammation and may lead to the development of novel therapies that will enhance patient quality of life and survival.

   Dr. Kazim Husain is maintaining our SHIP mouse colony and we reported earlier that our original breeder pairs reproduced 33 offspring. The team has obtained five male SHIP (KO) and three female SHIP Knock Out (KO) mice. Male SHIP (KO) mice will be kept on pairing with

SHIP+/- (Heterozygous (HET)) female and breeding further to get the required numbers of female SHIP (KO) mice for our experimental project work. The team is getting SHIP (KO) mice for our experiments however, SHIP Wild Type (WT) mice are lacking. As per the suggestion of our collaborator Dr. Margaret Hibbs, we started pairing (mating) SHIP (WT) male and female (young) mice as well as SHIP (WT) male and SHIP (HET) female mice to get SHIP (WT) mice.

Previously, the team reported that PC downregulated SHIP-1 expression in mice. However, what regulates SHIP-1 expression, has yet to be identified. miRNA-155 is one of SHIP-1 regulators. Our qRT-PCR preliminary results from the Bone Marrow (BM) of Heterotopic PC (HPC) mice showed an increase in miR-155 gene expression compared to the BM from control mice. In addition, the team observed that API treated HPC mice had a significant decrease in miR-155 gene expression compared to untreated HPC mice. Additionally, a significant reduction in SHIP-1 gene expression in the BM from HPC mice compared to control mice was observed. Interestingly, API treated HPC mice demonstrated a significant increase in SHIP-1 gene expression in the BM compared to untreated HPC mice. Next, the team asked if SHIP-1 expression correlates with SHIP-1 protein expression. Our immunofluorescence microscopy analysis shows that in the BM of HPC mice we observed a decrease in SHIP-1 compared to CTRL, however API treated HPC mice showed an increase of SHIP-1 BM compared to untreated BM from HPC mice. These results are also correlated with a decrease in the numbers of M-MDSC in the TME of HPC-API compared to HPC mice via Wright Giemsa staining. Moreover, through qRT-PCR analysis, the team observed that miR-155 gene expression in the pancreas/pancreatic tumor is significantly up-regulated in orthotopic PC (OPC) vs CTRL mice, and that API significantly decreases miR-155 gene expression. These results correlate with the increase in SHIP-1 gene expression in OPC-API mice vs OPC mice (data not shown). The team has now identified that API targets and suppresses miR-155 as a potential mechanism by which API restores SHIP-1 expression linked to improving MDSC homeostasis and reducing M2 TAM associated immunosuppression in the TME of mice with PC.

The team has found that murine Panc02 cells, subcutaneously (s.c.), injected in C57BL/6 SHIPKO mice grew PC tumors faster compared to SHIPWT mice. More importantly, preliminary flow cytometry data shows an increase in M2 TAM (pro-tumor) and a decrease in M1 TAM (anti-tumor) in the tumor microenvironment (TME) of SHIPKO-HPC compared to SHIPWT-HPC mice. Thus, using SHIPKO-HPC and SHIPWT-HPC mice the team can show the vital role of SHIP-1 in delaying tumor progression and its impact on the expansion of M2 TAM in the pancreatic TME. However, the team has not yet fully explored how SHIP-1 expression regulates M1 vs. M2 TAM development within pancreatic TME. Deficiency of SHIP-1, a negative regulator of PI3K/AKT signaling, significantly enhances the M2 phenotype macrophages. The loss of SHIP-1 expression converts PI (3,4,5) P3 to PI (PI 4,5) P2, which results in the constitutively AKT1 activation and polarization of M2 TAM. The team published that SHIP-1 deficient mice demonstrated hyper-phosphorylation of AKT. Also, in our PC mice, a reduction in SHIP-1 expression, hyperphosphorylation of AKT, was reported. It is our proposal that API inhibits PC-induced miR-155 which in turn causes the up-regulation in SHIP-1 expression via AKT signaling that promote M1 TAM in the pancreatic TME. Therefore, it is ideal to orthotopically transplant pancreatic tumors into SHIPKO and SHIPWT mice to identify explicitly SHIP-1/AKTs dependent signal transduction events that regulate M1 and M2 TAM development in the pancreatic TME. All of this new data along with the proposed model strongly suggest that SHIP-1 expression is important for halting M2 TAM (pro-tumor) vs. M1 TAM (tumoricidal) development in the TME impacting anti-tumor immune responses of PC mice. These current results have been submitted in part of NIH NCI RO1 grant application June 5, 2020. In addition, these results will be added to our research manuscript and submitted to the Journal of OncoImmunology in August 2020.

Follow-on Funding:
State of Florida; SHIP-1: A Potential New Molecular Target for the Treatment of Pancreatic Cancer; Tomar Ghansah PhD; $816,514.00.

Collaborations: The team is currently collaborating with Jason B. Fleming, M.D. from Moffitt Cancer Center (Department Chair of GI Oncology and Tampa, FL) and Jose Trevino, M.D. from the University of Florida (College of Medicine/Department of Surgery and Gainesville, FL) for the purpose of gaining access to patient-derived xenograft (PDX) mice. PDX mice will prove to be beneficial for our pre-clinical studies by providing more realistic outcomes for potential immunotherapy treatments. Also, Dr. Trevino is a co-PI on a grant (NIH NCI RO1 grant) we submitted June 5, 2020.

Margaret Hibbs, PhD from Monash University (Melbourne, Australia) has provided me with 3 breeding pairs of transgenic SHIP (HET) mice on a F7 C57BL/6 background and started breeding colony.

Kazim Husain, PhD, DABT, is a research scientist from Moffitt Cancer Center is collaborating with me as I breed my own C57BL/6 SHIP (KO) and SHIP (WT) mouse colony.

Gerald Krystal, PhD (College of Medicine/Department of Pathology and Laboratory Medicine, and Vancouver, BC) and Laura Sly, PhD (College of Medicine/Department of Pediatrics and Vancouver, BC) from the University of British Columbia provided transgenic and wild type mice for on-going experiments so that our team can submit our pending manuscript.

Krystal Villalobos-Ayala, MS, is a full-time Lead Research Technician in the PI's lab. Krystal is currently working on experiments to improve the statistical analysis for her first author revised manuscript (previously submitted to PLOS One) that will be submitted to Journal of Immunology.

Journals: Husain, K., Williamson, T. T., Nelson, N., and Ghansah, T. Protein Kinase 2 (CK2): A Potential Regulator of Immune Cell Development and Function. Submitted to *OncoImmunology* June 2020.

Patents: None at the time of reporting

James and Esther King Biomedical Research Program
Appendix N
Fiscal Year 2019-2020 Active Grants
Funding Year 2016-2017

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditures	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
7JK01	University of Miami	Helen M. Bramlett, PhD	$1,253,753	$1,152,206.77	$101,546.28	3/09/2017	2/29/2020	No	Yes	Pending*
7JK02	H. Lee Moffitt Cancer Center	Christine Chung, MD	$1,896,200	$1,147,700.00	$748,500.00	3/16/2017	2/28/2022	No	Yes	No
7JK03	University of Miami	W. Dalton Deitrich, PhD	$941,589	$863,123.50	$78,465.75	3/08/2017	2/29/2020	No	No	No
7JK04	H. Lee Moffitt Cancer Center	Jhanelle Gray, MD	$1,895,355	$1,137213.00	$758,142.00	3/25/2017	2/28/2022	No	No	No
7JK05	University of Florida	Zhihua Jiang, PhD	$1,422,150	$1,303,637.50	$118,512.50	3/07/2017	2/29/2020	No	No	No

*Received notification of follow-on funding but award has not been finalized.

1. **Grant #:** 7JK01 Whole Body Vibration Improves Stroke Outcome in Nicotine-Exposed Rats

   Principal Investigator: Helen M. Bramlett, PhD

   Organization: University of Miami

   Grant Progress Report: During this last year, the team performed experiments where reproductively senescent Sprague–Dawley female rats and middle-aged male rats were exposed to Transient Middle Cerebral Artery Occlusion (tMCAO) and randomly assigned to either Whole Body Vibration (WBV) or no-WBV groups. Our analysis of inflammatory proteins and brain tissue continued, as well as blood for Western blotting and cytokine assays at 24h after tMCAO in both males and females or 30 days after WBV/no-WBV animals in females. We observed that nicotine increases the systemic expression of pro-inflammatory cytokines IL- 18, M-CSF, and Tumor Necrosis Factor (TNF) alpha and reduces the anti-inflammatory cytokine G-CSF in both middle-aged males and female rats 24 hours after tMCAO. Post-stroke WBV for a month shows a decrease in systemic expression of pro-inflammatory cytokines IL-1b, IL-6, IL-18, IFN-g, TNF-a as well as an increases Vascular endothelial growth factor (VEGF) expression in both nicotine-exposed male and female tMCAO rats after 30 days of WBV treatment. WBV does however, seem to target different cytokines in males and females, therefore future studies can with a dual treatment of WBV and treatments targeting these cytokines may allow for better sex-specific treatment. The team also performed western blotting and mitochondrial enzyme activity experiments on cortical and muscle tissues collected from tMCAO-WBV or tMCAO-control rats. Post-stroke WBV improves cortical and muscle mitochondrial functions. We also assessed for irisin levels which is secreted from muscles in response to exercise and WBV has shown to increase irisin levels in the human body following exercise. Rats exposed to nicotine or saline and treated with either WBV or no-WBV treatment after tMCAO were allowed to survive for a month and then, cortical tissues were collected in order to examine Irisin protein levels by western blotting. Results of Western blotting demonstrated that WBV treatment in saline exposed brain increases Irisin protein levels as compared to no-WBV group. However, similar changes were not observed in the nicotine exposed brain. We observed no differences in Irisin protein levels between no- WBV and WBV groups in nicotine exposed rat brains. The team was in the process of continuing our studies in the NCE, but due to COVID-19 some of our studies were delayed. However, recently our studies have started again.

   Follow-on Funding: Veteran's Affairs; Post-stroke combination of therapeutic hypothermia (TH) and whole-body vibration (WBV) improve cognition in nicotine-exposed rats; Ami Raval; Pending.

   Collaborations: None at the time of reporting

   Journals: Kerr N, Dietrich WD, Bramlett HM, Raval AP. Sexually dimorphic microglia and ischemic stroke. CNS Neurosci Ther 2019 25:1308- 1317. PMCID: PMC6887716.

   Patents: None at the time of reporting

2. **Grant #:** 7JK02 Molecular Signatures of Immunotherapy Response and Improved Survival in Tobacco-Related Head and Neck Cancer

   Principal Investigator: Christine H. Chung, MD

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

Grant Progress Report: Aim 1: Determine the Mesenchymal subtype signature and T-cell receptor clonality as predictive biomarkers and the Tumor Immune MicroEnvironment (TIME) in a context of tobacco use in Head and Neck Squamous Cell Carcinoma (HNSCC). Enrollment for 45 patients has been completed for the proposed phase I/II clinical trial of cetuximab and nivolumab in Cohort A. The team determined progression-free survival and overall survival and found patients with no prior exposure to Program Death (PD-1) inhibitors were likely to benefit from the combination of cetuximab and nivolumab. The results were presented at the American Society of Clinical Oncology Annual Meeting in June 2020. In addition, we initiated a collaboration with Flatiron Health and obtained real-world clinical data from patients treated with either cetuximab alone (N=177) or nivolumab (or pembrolizumab) alone (N=666) during our trial enrollment period as contemporaneous cohorts. Currently, the team is conducting statistical analyses for a publication, and have enrolled 40 patients in Cohort B (total enrollment goal 43 patients) which is the first line rec/met cohort. To evaluate T-Cell Receptor (TCR) clonality as a predictive biomarker, an analysis pipeline has been established and the team performed preliminary analyses on 33 DNA samples from peripheral blood mononuclear cells and 11 DNA samples from tumors profiled by TCR repertoire sequencing via ImmunoSEQ. The team observed that the “partial response” patient group tended to have higher TCR diversity scores compared to progressive and stable disease groups. Additional biomarker studies proposed in this aim are currently ongoing.

Determine tobacco-specific genoproteomic changes that create immunosuppressive TIME. All experiments in Aim 2 have been completed and published in March 2020. Leveraging these data, we submitted a NIH R01 application in June, 2020 to continue the project to comprehensively evaluate the tobacco-related effects on the oral cancer ecosystem through integrated multi-omics approaches, identify novel therapeutic agents leveraging the oral cancer-specific TIME, and develop an oral cancer specific web portal to advance the field in tobacco-related cancer research.

Develop smartphone-based assessment of patient-reported outcomes related to immunotherapy and smoking in HNSCC. A total of 100 patients have been recruited to participate in the study, of which 90 have completed the study and 10 are still being followed longitudinally. Preliminary results were presented at the Annual Meeting of the American Association for Cancer Research (AACR) in April 2020. The Functional Assessment of Cancer Therapy-General was used to assess quality of life. Overall quality of life, emotional well- being, and functional well-being significantly improved while physical well-being and social well-being remained stable. In addition, the overall quality of life in patients who were treated with PD-1 inhibitors in context of smoking status was evaluated. Current smokers had the most improvements in quality of life given the PD-1 inhibitors. When the team evaluated each of symptoms, current smokers had more symptoms at the baseline including fatigue, cough, wheezing, weight loss, insomnia, anxiety, etc. compared to the never or former smokers. The team is planning on expansion of this cohort in a follow up study.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: Tobacco smoking is associated with the immune suppressive microenvironment in head and neck squamous cell carcinoma (HNSCC). Clinical Cancer Research. 2020 Mar

15;26(6):1474-1485.

Long noncoding RNA, LINC00460, as a prognostic biomarker in head and neck squamous cell carcinoma (HNSCC). Am J Transl Res. 2020 Feb 15; 12(2): 684-696.

Patents: None at the time of reporting

3. **Grant #:** 7JK03 The Therapeutic Effects of P7C3-A20 in Stroke

   Principal Investigator: W. Dalton Dietrich, PhD

   Organization: University of Miami

   Grant Progress Report: During this last year, there were some issues with the laser and the team worked on re-establishing the model with another laser. Our previous Argon laser was unrepairable. As mentioned previously, our use of the cytovene pumps was not working as well, so use of another protocol for ablating neurogenesis in our stroke model was adopted. We will be using the anti-mitotic drug Ara-C which will be infused via Alzet miniosmotic pump into the posterior lateral ventricle to inhibit cellular proliferation in the dentate gyrus. It has taken some time to re-establish the model, but recently the team made progress on this protocol. Due to COVID-19, the work had to be discontinued, but the team has since started back re-verifying the infarcts. Once this is done, the team will proceed with using the anti-mitotic drug (ARA-C) that will knockdown neurogenesis similar to how the cytovene pumps were proposed. Some key personnel have left the university, but they have been replaced and there have been no delays due to personnel changes. The team anticipates being able to finish our studies during the NCE.

   Follow-on Funding: None at the time of reporting

   Collaborations: None at the time of reporting

   Journals: None at the time of reporting

   Patents: None at the time of reporting

4. **Grant #:** 7JK04 Targeting Immunosuppressive Cancer Associated Fibroblasts and Immune Checkpoints in NSCLC

   Principal Investigator: Jhanelle E. Gray, MD

   Organization: H. Lee Moffitt Cancer and Research Institute, Inc.

   Grant Progress Report: The field of lung cancer is rapidly evolving, however the standard for treating Non-Small Cell Lung Cancer (NSCLC) based on previous trials (KEYNOTE 21G, KEYNOTE 407, and KEYNOTE 189) is triplet combination therapy with platinum doublet plus pembrolizumab. This trial challenges the current landscape by removing chemotherapy and utilizing a novel triplet immunotherapy approach. From July 2019 – June 2020, the clinical study has seen substantial progress in concurrence with its aims, goals, and objectives.

The first aim of this study was to establish the safety and efficacy nivolumab plus/minus nintedanib in both immunotherapy-naïve and pre-treated patients with advanced NSCLC. The Phase I trial sought to successfully identify safe dosing levels including Maximal Tolerated Dose (MTD) and Dose Limiting Toxicity (DLT). Phase I successfully reached accrual resulting in the completion and approval of a Phase I Summary Report by the Internal Protocol Monitoring Committee. Results identified a recommended safe dose of orally administered 150 mg Nintedanib daily. Establishment of recommended safe dose allowed study progression into phase II dose expansion which commenced on May 29, 2019.

Despite COVID 19, diligent efforts have been made to screen, consent, enroll patients into the dose expansion phase. During this reporting period, phase II has a cumulative total of 106 referrals, 45 patients consented, and 24 patients enrolled (Arm A: 7; Arm B: 17). Pathology has established logistics and quality control of appropriate specimen collection from enrolled patients, established communications with Moffitt's tissue core, CLIA microscopy core, and the mathematical modeling group to plan for biomarker analysis of collected tissue. Pathology is also synthesizing plans for biomarker testing of specimens collected by trial to evaluate for tumor infiltrating lymphocytes, Program Death Ligand (PD-L1), and genetic markers, such as FGFR mutations, for correlation with clinical response and resistance. Appropriate specimen collection and processing was implemented for flow cytometric analysis of peripheral blood immune phenotype which continued despite Covid19. In addition, the remainder of Phase I patient cohort samples (Patient 001, 002, 003, 006, 007, 009, 010, 011, 012, 013, 014, 015, 017, 018) are currently being analyzed for their immune phenotype including the maturation status of T-cells and expression of immune checkpoints and co-stimulatory molecules. Culmination of these efforts satisfy Aims 2 and 3 of the study which include correlative biomarkers and resistance mechanisms in NSCLC patients. Culmination of these efforts resulted in the presentation of a "Phase I Study of Nivolumab and Ipilimumab Combined with Nintedanib in Advanced NSCLC" at the World Conference on Lung Cancer (WCLC) in Barcelona, Spain; September 2019.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

5. **Grant #:** 7JK05 Mechanisms for Tobacco Smoke to Modulate Aortic Aneurysm Development

Principal Investigator: Zhihua Jiang, PhD

Organization: University of Florida

Grant Progress Report: Tobacco users continue to suffer five to six times greater risk of developing aortic aneurysms than their non-smoking counterparts. Unfortunately, the rate of ever and current use of tobacco is the highest in Florida, and higher than in any other state nationwide. Although the risk of smoking has been recognized for decades, medical treatments to control the risk remain not available due to poor understanding of the mechanisms through which smoking exacerbates aortic aneurysm formation. In the past year, research staff of this project continued approaching to the goals that were laid out during the initial submission.

These include: to develop an animal model capable of reliably recapitulating the exacerbating effect of cigarette smoke; to evaluate how disruption of the immune function, which occurs frequently in smokers, exacerbates the pathogenesis and progression; and to test the therapeutic potential of strategies aimed at correcting the disrupted immune function. In the last reporting period, project staff documented their observations that shifting of the immune polarization to the Th2 (type 2 T-helper) end by adoptive transfer of ex vivo expended T-cells or serological abolishment of Th1 (type 1 T-helper) polarization exacerbated dilation of aortic aneurysms. Excited by this discovery, research staff employed a genetic approach to evaluate implications of this phenomenon in aortic aneurysm development. T-bet is a transcriptional factor essential for T-cells to acquire a Th1 lineage identity and encoded by the gene named Tbx21. Mice null for Tbx21 will mountain a Th2 prominent immunity when challenged with inflammatory stimuli. Using this transgenic mouse strain, several experiments were performed to evaluate the effect of Th2 polarization on aortic rupture and dilation, with the inflammatory response in the developing aneurysms characterized with flow cytometry and cytokine profiling analyses. As documented in the previous reports, the results showed that Tbx21 deficiency exacerbated early aortic dilation and promoted aortic rupture. Mechanistically, exaggeration of the aneurysmal degeneration in Tbx21 null mice is associated with a higher percentage of Th2 cells, a higher level of the Th2 signature cytokines, and a greater amount of Matrix MetalloProteinase 2 (MMP2) activity in the aneurysms. Additionally, research staff compared infiltration of T-cells in aortic aneurysms treated with and without nicotine. The results showed that exposure to nicotine significantly enhanced accumulation of T-cells in the aneurysms. These findings are consistent with the previous observations made by the project staff with adoptive transfer of T-cells and serological abrogation of Th1 differentiation and have provided further evidence to support our initial hypothesis that a skewing of the Th1/Th2 balance to Th2 predominance underlies tobacco smoke-exacerbation of aortic aneurysms. Currently, project staff are working on experiments to characterize the phenotype of inflammatory cells and local cytokine milieu in aortic aneurysms exposed to nicotine treatment. A manuscript describing the successful creation of a mouse model for investigation of the exacerbation of aortic aneurysm formation by nicotine is in preparation and will be submitted for publication in a few weeks.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

James and Esther King Biomedical Research Program
Appendix O
Fiscal Year 2019-2020 Active Grants
Funding Year 2015-2016

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
6JK02	H. Lee Moffitt Cancer Center	Vani N. Simmons, PhD	$1,186,164	$1,028,008.80	$158,155.20	3/19/2016	2/28/2021	No	Yes	No
6JK04	Florida International University	Maria Jose Miguez, MD	$1,628,449	$1,411,322.47	$217,126.53	3/19/2016	2/28/2021	No	Yes	No

1. **Grant #:** 6JK02 Facilitating Smoking Cessation with Reduced Nicotine Cigarettes

Principal Investigator: David J. Drobes, PhD to Vani N. Simmons, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Grant Progress Report: This project is testing a novel smoking cessation intervention that utilizes reduced nicotine content cigarettes prior to quitting, along with targeted self-help treatment materials and individual counseling. The overall purpose is to determine if this smoking cessation strategy is efficacious, and whether an immediate or gradual transition to very low nicotine content cigarettes during the pre-quit period is optimal. The primary work completed during this reporting period has been the continuation of Study 2 participant recruitment and data collection. The study team initiated a social media campaign which resulted in substantial progress in participant recruitment and data collection during this reporting timeframe. Research staff reports the following numbers to date: 115 randomizations, 629 telephone eligibility screenings, 632 study visits, 76 treatment completers, 60 two-month follow-up assessments, and 31 six-month assessments. Study 2 data is entered regularly, and the study database is reviewed monthly by our statistician. Moreover, the study team submitted an article describing the process of developing the intervention, pilot study results, and modifications made prior to the main Randomized Controlled Trial (RCT). This article also describes the study design and planned analyses for the RCT.

Due to COVID-19, our institution has applied policies that have impacted the recruitment and intervention activities of the project. A summary is provided below:

- All in-person recruitment and visit procedures that require in-person interaction (including weekly distribution of study cigarettes) have been halted at Moffitt Cancer Center until further notice.
- Follow-up of study participants that can be conducted online/remotely is ongoing. This includes final treatment visits (not including distribution of study cigarettes) and follow-up sessions. These measures were implemented in March and were approved by the University of South Florida (USF) Institutional Review Board and the FDA. To re-initiate recruitment and intervention activities, we plan to progressively integrate in-person interactions in line with institutional, local, and federal guidelines. We have recently submitted a request for the following protocol modifications:
- Participants may visit the facility to conduct physiological assessments (CO, BAL, pregnancy, toxicology, heart rate, and blood pressure), complete questionnaires for the screening visit, and receive/return study product at subsequent visits. BAL and CO devices will be sanitized after each use and the study team will alternate devices between participants.
- Participants may complete questionnaires, be consented, and counseled virtually (e.g., via Zoom), to minimize face-to-face interactions when at the facility.
- To maximize retention, study personnel may use third-party directory services (i.e., whitepages.com) if they are unable to locate participants.

These changes will be submitted to the USF Institutional Review Board for approval and subsequently reported to the FDA.

<u>Impact to Floridians:</u>
As we have not completed recruitment for Study 2, sufficient data are not yet available to determine the impact to Floridians. Nevertheless, cigarette smoking remains the top avoidable

cause of death in Florida. The current study has the potential to validate a novel smoking cessation method that could be part of an effort to reduce the burden of disease and death from smoking in Florida and beyond.

Follow-on Funding: None at the time of reporting

Collaborations: University of South Florida (Tampa, FL). Twelve undergraduate students received training and participated on the project (as Research Interns) during this reporting period.

University of South Florida, Department of Psychology (Tampa, FL). One clinical psychology graduate student received training and participated on the project (as a smoking cessation counselor) during this reporting period: Leslie Sawyer

University of Alabama (Birmingham, AL). One visiting research scholar participated on the project during this reporting period: Abdullah Alanazi

University of Santiago de Compostela (Spain). One visiting research scholar participated on the project during this reporting period: Maria Barroso

Journals: Conn, M.R., Brandon, T.H., Lorenzo, Y.L., Sawyer, L.E., Simmons, V.N., Sutton, S.K., Donny, E.C., Hatsukami, D., and Drobes, D.J. (manuscript submitted). Facilitating Smoking Cessation Using Reduced Nicotine Cigarettes: Intervention Development and RCT Study Design.

Patents: None at the time of reporting

2. Grant #: 6JK04 Biobehavioral Intervention for Smokers Living with HIV

Principal Investigator: Maria Jose Miguez, MD, PhD

Organization: Florida International University

Grant Progress Report: The scope of the burden of disease and death that cigarette smoking imposes on the public's health is extensive. Moreover, additional threats are emerging because smoking entails harms for some subpopulation groups. For example, among people living with HIV, who are nowadays more likely to die from a tobacco-related disease than from HIV.

Our ongoing study is one of the largest tailored clinical trials designed to address smoking among people living with HIV. The goals are first to reduce smoking rates and amounts of smoking per day in 500 individuals, most of whom are minorities. Although final numbers will only be available at the end of the clinical trial, data suggest that our team has obtained quitting rates like the ones obtained in the general population, and therefore significantly higher than those obtained in prior studies with this population. Although quitting smoking has been shown to have a negative impact on body weight and is thought to be a frequent deterrent of quitting smoking, our intervention demonstrated that this can be avoided, or at least reduced, with the use of nicotine replacement therapy. Even though some studies have observed that quitting smoking may impact glucose metabolism, such information has not been previously validated in a cohort of people living with HIV. However, it will be highly valuable to determine which smokers are most likely to develop alterations in glycemic control and to identify possible

underlying mechanisms. Such information could be useful in tailoring preventive interventions. Therefore, as a secondary goal the team valuated changes in blood glucose during the intervention. It is important to analyze the effect by type of cigarette because: a) approximately two-thirds of HIV infected smokers prefer mentholated cigarettes, and b) whether smokers of mentholated cigarettes have more or less homeostatic changes than smokers of non-mentholated cigarettes are currently unknown. We demonstrated a close relationship between smoking and obesity, particularly among individuals using mentholated cigarettes. In addition, we documented the prospective associations between smoking and glycemia levels, particularly among minorities. Since we demonstrated that quitting smoking alters glucose levels, we can assume that our baseline observations imply more than a simple association. Such information is critical because by inducing obesity and poor glycemic control, smoking can boost the risks of morbidity and mortality among people living with HIV, and thus slash the gains acquired with antiretroviral therapy. Given these findings and the widespread use of menthol as an additive on e-cigarettes and hookah additional studies are justified. Since growing evidence indicates that individuals with reduced pulmonary function, obesity, and diabetes are at risk of developing incident COVID-19 disease, it is now essential to address the main modifiable risk factor of smoking in order to prevent the development of its complications.

Moreover, increased efforts to reduce smoking rates is needed now more than ever in Florida as we are one of the main epicenters of the COVID-19 pandemic. Smoking cessation programs will be very helpful for the prevention and management of the COVID-19 disease because additional waives of SARS-COv-2 are expected.

Follow-on Funding: None at the time of reporting

Collaborations: Actively collaborating with the University of Miami. Dr. Castro, a University of Miami faculty, is the study physician of the clinical trial.

The study procedures are administered at the Clinical Research Center, which is staffed by professionals with experience in clinical research involving PLWH.

Performing laboratory testing at the University of Miami facilities. This laboratory was selected because they take part in the national (NIH, CDC) external quality proficiency testing programs.

Journals: Miguez MJ, Bueno D, Quiros C. (2020) Health Disparities on COVID-19: The need of a Holistic Model that Must Recognize the Biology Perspective. New England Journal of Medicine.

Miguez MJ, Bueno D, Quiros C. (2020) What We Do Not Know About Smoking and COVID-19 Can Hunt Us Down. Tobacco Control.

Miguez MJ, Bueno D, Quiros C. (2020) Gender differences in smoking behaviors are narrowing but health impact is wider in people living with HIV. Journal of gender differences.

Patents: None at the time of reporting

James and Esther King Biomedical Research Program
Appendix P
Fiscal Year 2019-2020 Completed Grants
Funding Year 2015-2016

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
6JK01	H. Lee Moffitt Cancer Center	Wei, Sheng MD	$1,231,336	$1,202,816.92	$28,519.08	3/19/2016	8/31/2019	Pending*	Yes	Yes
6JK03	University of Florida	Liao, Daiqing, PhD	$795,236	$793,969.77	$1,266.23	3/09/2016	8/31/2019	Yes	Yes	No
6JK06	H. Lee Moffitt Cancer Center	Jong Y.Park, PhD	$1,231,336	$1,162,928.40	$68,407.60	3/21/2016	8/31/2019	No	No	Yes
6JK08	Florida Atlantic University	Jang-Yen Wu, PhD	$ 1,231,336	$1,231,336.00	$0.00	3/31/2016	8/31/2019	Yes	Yes	Yes

*Patents have been filed but have not been approved yet.

1. **Grant#:** 6JK01 Nanoparticle-Based Targeting of miR183 for Immunotherapy of Lung Cancer

   Principal Investigator: Sheng Wei, MD

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

   Grant Progress Report: The main goal of this proposal was to provide proof-of-concept that targeting Tumor Growth Factor (TGF) or miR183 can restore Natural Killer (NK) function in vivo against lung cancer, using NSG mice implanted with human A549 lung tumors. For the most part, we have been able to observe thus far the proof of our initial hypothesis in vitro in primary NK cells. First, the formulation of proposed Poly Lactic-co-Glycolic Acid (PLGA) particles was changed due to unforeseen circumstances including the uptake by NK cells which was solved by the use of MnO2 nanoparticles which are smaller and readily uptaken by NK cells. Research staff saw that while the NK cells are not as resilient to NP treatment as NK92, demonstrated by a shift in their scatter properties, they are alive and able to reduce the expression of TGFbR2 after NP uptake. In the *in vivo* model of the project there were unforeseen challenges, but they have provided a clear indication of why there may be a tumor-induced restriction of NK cells and other cytotoxic cells from the tumor bed. While the team will continue in the process of corroborating and expanding our collaboration into the use of nanoparticle delivery into NK cells, the in work produced in the in vivo model has shed important information that the Wei lab has also been using to extrapolate to other more commonly available therapies like CAR-T functionality. This work has led to a potential publication which the Wei lab will soon submit for publication. On the other hand, it has allowed us to continue testing the role of NK cells treated with specific nanoparticles and how they behave in vitro. We have also taken advantage of our collaboration to start including other targets into the nanoparticles to modulate the cytotoxic activity of NK cells against tumors. The main issue was that primary NK cells were not migrating inside the tumor (our main target site) even though they were making it to hematopoietic tissues. Research staff overcame that issue through the development of CX3CL1 overexpressing A549 and H1299 cells, or as a proof-of concept, overexpressing its receptor on TILs stimulating the migration of these cells into the tumor and expanding that concept beyond the nanoparticle study for the potential benefit of lung cancer patients.

   Summary of most significant accomplishments for Aim 1: Develop human lung-xenograft immunotherapy model in NSG mice. Successful establishment of a murine model using IL-15 pre-activated NK cells 24h prior to in vivo injection, supplemented with IL-15 during injection into NSG mice bearing CX3CL1-A549 tumor cells. This allows the intra-tumoral infiltration of primary NK cells. Will confirm the results from the migration of primary NK cells post injection into the tumor bed. Successful in vitro uptake of labeled particles and selection of optimal dose for NK cell survival and uptake efficiency. Successful reduction of TGFbR2 in the surface of primary NK cells. Tested the in vitro functionality of siRNA loaded NP in primary NK cells in cytotoxic assays. Successful testing of lung xenograft model with cytotoxic CAR-T cells and preparation for NP loaded therapeutic strategies.

   Summary of most significant accomplishments to date for Aim 2:
   Engineering Biomimetic Tumor Models to Interrogate NK Cell-Cancer Cell Interactions

   - Engineered synthetic PolyEthylene Glycol (PEG)-based hydrogels with controlled presentation of cell adhesion sites and enzymatic degradation sites that support NK cell migration, maintain different immunosuppressive phenotypes of two lung cancer cell lines, which allows for studying NK cell-cancer cell interactions. Demonstrated that NK

cell infiltration into the tumor model depends on the stage of tumor growth and metastatic and immunosuppressive phenotype of the cancer cells. The more immunosuppressive phenotype in the H1299 tumor models and 'late' stage tumor models may explain the reduced NK cell infiltration. The decreased function also corresponded to inhibition of NK cell function by these tumor models, relative to the A549 and 'early' stage models. The role of TGF-β on NK cell migration was further evaluated by inhibiting TGF-β receptor I (TGFBRI) in NK cells, then studying their infiltration into the tumor models, which was greatly increased relative to control NK cells. Engineered 3D tumor models recapitulated key feature of NK cell suppression and may provide useful tools to develop and test new cancer immunotherapies.

Nanoparticle development for delivery of antimiR183 to NK cells

- The team proposed to use poly lactic-co-glycolic acid (PLGA)-based nanoparticles to deliver miRNA to NK cells, as we had previous success with PLGA NPs for gene delivery to tumors. The PLGA NPs demonstrated good cytocompatibility with the NK cells. However, initial attempts at nucleic acid delivery to NK cells using PLGA were not successful.
- Established a new particle formulation of Manganese dioxide (MnO_2) with a cationic polymer coating and PEG modification. These particles are stable at small sizes (15-30mn) and are able to complex efficiently with nucleic acids.
- Successful gene knock-down in NK cells by siRNA-loaded MnO_2 NPs, specifically for siRNA for TGFBR2, confirmed by gene expression and immunofluorescent staining, which recovered NK cell function against cancer cells.

Nanoparticle and NK cell injection into NSG mice

- Established a staining method (DiR) and injection protocol (~6 million cells) to result in NK-92 cell (+IL-2) tumor localization after I.V. injection into A549 tumors (no modification to the tumor).
- Applied the staining protocol to image NK-92 cell (+IL-2) (~6 million cells) tumor retention after intra-tumoral injection into A549 tumors (no modification to the tumor).

Follow-on Funding: National Science Foundation; "CAREER: Engineered tumor models to study the recruitment and activation of Natural Killer cells"; Sheng Wei MD; $550,000.

Collaborations: None at the time of funding

Journals:

Trinh T, Kandell W, Tu N, Adams WA, Gilvary D, Cheng P, Chen X, Chen Y, Eksioglu EA, Djeu J, Sharma B, Wei S. Preparing for submission in 2019. On murine CX3CL1 model of immune restriction

Adjei I, Jorden J, Tu N, Trhin T, Kandell W, Tu N, Wei S, Sharma B, Functional Recovery of NK Cell Activity by Nanoparticle- mediated Delivery of TGFBR2 siRNA, Journal of Interdisciniplanry Nanomeidicine. August 2019

Temples M., Adjei, I., Nimocks P., Djeu, J., Wei, S., Sharma, B., Engineered Three-Dimensional Tumor Models to Study Natural Killer Cell Suppression, in review, ACS Biomaterials Science and Engineering

Patents: Nanoparticles for Intracellular Drug Delivery to Natural Killer Cells, Provisional Patent Filed February 20, 2018, Serial No. 62/632,922

Composition and method for targeting natural killer cells in immunotherapy to overcome tumor suppression with manganese dioxide nanoparticles, International Patent Application Number PCT/US19/18677, filed February 20, 2019.

2. Grant#: 6JK03 Pharmacologic Inhibition of Acetyltransferase CBP/p300 as a New Therapeutic Approach for Breast Cancer

Principal Investigator: Daiqing Liao, PhD

Organization: University of Florida

Grant Progress Report: CBP/p300 are promising therapeutic targets for breast cancer and other cancer types (e.g., p300 in prostate cancer). Scientists in academia and pharmaceutical industry have made progress in discovering lead CBP/p300 inhibitors. CCS1477, a potent and selective p300/CBP bromodomain inhibitor, has been advanced to phase 1 and phase 2 clinical trials for treating metastatic castration-resistant prostate cancer (ClinicalTrials.gov Identifier: NCT03568656). Another promising lead compound (A-485) that potently and specifically inhibits the catalytic activity of p300/CBP as an acetyltransferase was identified in 2017 (Lasko LM et al., 2017). As reported previously, we have identified novel compounds that inhibit CBP/p300. We had collaborated with researchers at Sanford Burnham Prebys Medical Discovery Institute at Lake Nona, FL, in an effort to optimize potency and drug properties of these compounds. Unfortunately, funding was not renewed for the planned chemical optimization efforts. Nonetheless, as reported previously, we had continued our efforts to characterize the new EP series of analogs and identified promising compounds with potent CBP/p300 inhibitory effects. Additionally, we had further characterized SR-2210, a lead CBP/p300 inhibitor proposed in our original application. Using a more quantitative proteomic approach, we found that SR-2210 exhibits specific inhibition on the acetylation of histones and other known CBP/p300 protein substrates. The research team validated shRNA clones for specific genetic depletion of EP300 and CREBBP. Our findings showed that CBP/p300 depletion sensitized SR-2210 and EP compounds. Furthermore, SR-2210 sensitized drug-resistant breast cancer cells to endocrine therapy. Research staff validated an anti-CBP antibody for ChIP experiment and conducted pilot in vivo experiment which successfully generated xenograft PDX tumors.

As reported previously, the team compared SR-2210 and EP compounds with A-485. Our data showed that A-485 was highly potent and specific for inhibiting catalytic function of p300/CBP. As reported below, SR-2210 and EP compounds were less potent p300/CBP inhibitors than A-485. Significantly, A-485 strongly downregulated ERα expression at both the mRNA and protein levels. ERα target genes such as MYC and CCND1 (encoding cyclin D1) were significantly downregulated. mRNA profiling experiments show that A-485 suppressed ERα signaling and other p300/CBP-regulated pathways such as the aryl hydrocarbon receptor (AHR) signaling, which was shown recently to be dependent on p300/CBP (Weinert B et al. 2018). Using chromatin-immunoprecipitation (ChIP) assay, we found that A-485 reduced the recruitment of p300 to the MYC promoter as well as the levels of acetylated lysine 27 of histone H3 (H3K27ac) at the MYC promoter, which is specifically catalyzed by p300/CBP. These data are consistent with our original scientific premise that pharmacologic CBP/p300 inhibition is a promising therapeutic strategy for treating breast cancer, especially the ER+ subtypes. Because A-485 is

clearly superior over SR-2210 and EP compounds in inhibiting p300/CBP, we had used A-485 to assess the mechanism by which pharmacologic p300/CBP inhibition blocks ERα signaling and tumor growth during the 6-month extension period.

As summarized below, our findings have provided the preclinical proof-of-concept that targeting CBP/p300 can potentially be effective to block the ERα signaling pathway for treating advanced ER+ breast cancer. Our findings are significant in that they provide scientific basis for developing novel treatments targeting p300/CBP. Such treatments may lead to better clinical outcome for patients with advanced ER+ breast cancer, which accounts for most patient deaths due to breast cancer.

Follow-up Funding: None at the time of funding

Collaborations: None at the time of funding

Journals:
Two-way Horizontal and Vertical Omics Integration for Disease Subtype Discovery. Z Huo, L Zhu, T Ma, S Han, D Liao, J Zhao, G Tseng. Statistics in Bioscience (SIBS). May 2019.

DAXX in cancer: phenomena, processes, mechanisms, and regulation. Mahmud I, Liao D. *Nucleic Acids Research.* July 2019.

DAXX drives de novo lipogenesis and is a novel target for cancer therapy. Mahmud I, Tian G, Wang, J, Huo Z, Lewis J, Waddell A, Zhao LY, Li JL, Garrett T, Daaka Y, Liao D. *Nature Communications (under review).* October 2019.

Patents:
Provisional U.S. Patent Application; Title: POLYPEPTIDE INHIBITOR OF DE NOVO LIPOGENESIS IN CANCER CELLS; Serial No: 62/653, 183. Filing Date: 4/05/2018 Inventors: Daiqing Liao, Iqbal Mahmud, Guimei Tian UF#-17034 (222110-8200)

International Patent Application. Patent Application Serial No. PCT/US2019/026011, filed 04/05/2019; Title: POLYPEPTIDE INHIBITOR OF DE NOVO LIPOGENESIS IN CANCER CELLS; Applicant: UNIVERSITY OF FLORIDA RESEARCH FOUNDATION Inventor(s): Daiqing Liao, Iqbal Mahmud, Guimei Tian Ref No.: UF#-17034 (222110-8200)

3. Grant#: 6JK06 Biobank for African American Prostate Cancer Research in Florida

Principal Investigator: Jong Y. Park, PhD

Organization: H. Lee Moffitt Cancer Center

Grant Progress Report: Prostate cancer disproportionally affects men of African Ancestry (AA) who have much higher incidence and mortality rates than Caucasian men. In the state of Florida, approximately 1,700 AA cases were reported every year according to the Florida Cancer Data System (FCDS) of the Florida Department of Health.

We proposed to build a statewide biobank to support prostate cancer research among men of African Ancestry in Florida. It has not been initiated due to various reasons, such as limited resources to establish the infrastructure for collaborative data and biospecimen

collection.Our proposed project generated the development of an extremely valuable research asset for health disparity studies for prostate cancer. This resource allowed researchers to leverage additional national funding, such as NIH, will contribute to generate important scientific findings and ultimately lead to better strategies to reduce prostate cancer incidence and mortality. Indeed, this FL biobank project leaded to be a part of NIH supported U19 grant, titled, Research on Prostate Cancer in Men of African Ancestry: Defining the Roles of Genetics, Immunity and Stress Study (RESPOND).

After we received the award in March 2016, an IRB approval was obtained from the Florida Department of Health (DOH) on Oct. 19th 2016 and received requested patients' data on Jan. 18th 2017. The data included mailing address and telephone information and their epidemiological and clinical data of 7,959 African American prostate cancer patients who was diagnosed since Jan 2013 in Florida. We obtained IRB approvals for continuation in October 2017, October 2018, and October 2019.As of Oct. 25th 2019, we received information of a total of 7,959 AA PCa cases during the ascertainment period from the Florida Cancer Data Registry. Among AA cases who sent information packets, a total of 305 were found to be ineligible mainly due to deceased patients. 1,561 declined ether by mail or phone, 1,773 could not be located, and 3,759 'have not responded to the initial information packet/phone after total of 5 attempts. Total of 561 patients were signed informed consent.

For Aim 2, we performed proposed epigenetic analysis using tumor samples collected under this project. We analyzes DNA methylation profiles between tumor vs. normal, aggressive vs, indolent types with prostate tumor tissues we collected. We extracted 161 tumor and normal DNA from AA cases FFPE blocks and performed epi genomewide methylation analysis.

With active continuation protocol, we will analyze data, prepare manuscripts and publish articles. In addition, this FL biobank study for AA men leaded Moffitt Cancer Center to be a part of U19 NCI grant, proposed to recruit AA prostate cancer patients in 9 institutes from 7 States. The title of grant is Research on Prostate Cancer in Men of African Ancestry: Defining the Roles of Genetics, Immunity and Stress Study (RESPOND) (PI: Chris Haiman (Univ. Southern California)).

Follow-on Funding: National Cancer Instittue, $27,000,000

Collaborations: None at the time of funding

Journals: None at the time of funding

Patents: None at the time of funding

4. **Grant#:** 6JK08 Granulocyte Colony-Stimulating Factor (GCSF) Gene Therapy for Stroke

   Principal Investigator: Jang-Yen Wu, PhD

   Organization: Florida Atlantic University

Grant Progress Report: The research team has established the mouse Bilateral Carotid Artery Occlusion (BCAO) stroke model and demonstrated enhanced survival rate of BCAO mice through treatment with GCSF protein; the team has demonstrated enhanced protection by a combination of G-CSF and Sulindac in PC12 cells subjected to glutamate excitotoxicity, a major underlying mechanism of stroke-induced neuronal injury. The following expression vectors have been constructed containing GCSF gene for expression of the mRNA for GCSF: The CMV promoter containing vector driving constitutive expression of GCSF, namely AAV- CMV-GCSF) The neuron Specific Synapsin-1 (SYN-1) promoter containing vector namely AAV- SYN-1-G-CSF designed to ensure expression of GCSF at the neuronal site and prevent possible detrimental effects of non-specific expression of GCSF) A hypoxia responsive element (HRE) promoter domain plus the minimal CMV promoter namely: AAV-HRE-CMV-GCSF which is regulated under hypoxia for restricting expression to the ischemic regions only); The vector including HRE and synapsin promoter domains namely AAV-SYN-1-HRE-GCSF, which is designed to regulate expression of GCSF by hypoxia and specifically at the neuronal site; a corner test for behavioral analysis of BCAO mice with or without GCSF treatment has been established. The locomotor activity behavioral test has been established in addition to corner test and shown improvement of locomotor activity in mouse BCAO stroke animal model treated with GCSF. The research staff has demonstrated reduction of autophagy-mediated neuronal death in BCAO animal model after treatment with GCSF. The synergistic effect of GCSF and taurine combination therapy in protecting PC12 cells under hypoxic conditions has been shown. Significant progress of the project has been made in the quarter from 10/01/2016-12/31/2016 in the following three areas: The team demonstrated protection of PC12 cells against cell death from hypoxia/re- oxygenation using AAV-granulocyte GCSF gene therapy and/or GCSF protein. The team has demonstrated neuroprotection of GCSF therapy in bilateral carotid artery occlusion (BCAO) mouse stroke model as demonstrated in changes of mitochondrial stress biomarkers, Drp1, p53, and OPA1 using immunohistochemical analysis and by behavioral tests. The team demonstrated neuroprotection of GCSF gene therapy in bilateral carotid artery occlusion (BCAO) mouse stroke model as demonstrated in changes of apoptosis and endoplasmic reticulum (ER) stress biomarkers including GRP78, pIRE1, XBP1, ATF4 and Bax by immunoblotting analysis. Significant progress has been made of the project in the quarter from 04/01/2017-06/30/2017 in the following five areas: A bilateral carotid artery occlusion (BCAO) stroke model in mice with additional verification monitoring the cerebral blood flow with a Laser Doppler Flowmeter has been established. The team has demonstrated neuro-protection of GCSF gene therapy in the BCAO mouse stroke model as demonstrated in decrease of Endoplasmic Reticulum (ER) stress markers, GRP78, pIRE1 and XBP-1. Neuroprotection of GCSF gene therapy in the bilateral carotid artery occlusion (BCAO) mouse stroke model as demonstrated in decrease of autophagy stress marker, Beclin1. Neuroprotection of GCSF gene therapy in the bilateral carotid artery occlusion (BCAO) mouse stroke model as demonstrated in decrease of DRP1, a marker of mitochondrial stress, and increase of OPA1, a marker of mitochondrial enhancer. Neuroprotection of GCSF gene therapy in the bilateral carotid artery occlusion (BCAO) mouse stroke model as demonstrated in the locomotor activity test. Significant progress of the project in the quarter from 07/01/2017-09/3/2017 in the following five areas: Delivery of AAV-hGCSF gene vectors to mice via eye drop method and demonstration of expression of hGCSF protein in the brain. Delivery of AAV-hGCSF gene vectors to mice via eye drop method and determination of their effect on locomotor activity in BCAO mouse model. Protection of hypoxia-induced toxicity in PC-12 cells by AAV-CMV-GCSF vector. Protection of Aβ-induced toxicity in PC-12 cells by GCSF. GCSF gene demonstrates strong neuroprotective action against A-Beta (Aβ) toxicity on PC-12 cell cultures.

Follow-on Funding:

Postdoctoral Fellowship; Jigar Modi; $100,00.

Collaborations:
Collaboration with Dr. Yunging Kang, Assistant Professor, Department of Ocean and Mechanical Engineering, Florida Atlantic University, Boca Raton, FL.

Collaboration with Dr. Yunging Kang, Assistant Professor, Department of Ocean and Mechanical Engineering, Florida Atlantic University, Boca Raton, FL.
Journals:
G-CSF Attenuates Neuroinflammation and neuronal Apoptosis via mTOR/p70S6K Signaling Pathway in neonatal Hypoxia-Ischemia model rat. John Sieh Dumbuya; Lu Chen; Si Yun Shu; Lin Ma, Wei Luo, Fei Li, Jang- Yen Wu, Bin Wang. Scientific Reports. Submitted 8/15/2019.

Granulocyte-colony Stimulating Factor Gene Therapy as a Novel Therapeutics for Stroke in a Mouse Model. Janet Menzie- Suderam, Jigar Modi, Hongyaun Xu, Andrew Bent, Paula Trujillo, Kristen Medley, Eugenia Jimeneza, Jessica Shen, Rui Tao, Howard Prentice, Jang-Yen Wu. Neurotherapeutics. Submitted 8/9/2019.

Mode of Action of Granulocyte-colony Stimulating Factor (G- CSF) as a Novel Therapy for Stroke in a Mouse Model. Jigar Modi, Janet Menzie-Suderam, Hongyuan Xu Paola Trujillo, Kristen Medley, Rui Tao, Howard Prentice, Jang-Yen Wu. Journal of Biomedical Science. Submitted: 08/05/2019.

AEURA, a novel homeopathic agent, shows high level protection against viral infection and stress induced neuronal toxicity. Prentice H and Wu J.-Y. Journal of Biomedical Science and Applications. 2018.

Taurine 11. Hu, J., Piao, F., Schaffer, S.W., El Idrissi, A., Wu, J-Y. Advances in Experimental Medicine and Biology 1155. 2019.

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Patents: Title:
TREATMENT FOR ISCHEMIC STROKE; U.S. Patent Number: 9,050,305 Date issued: June 9, 2015; Inventors: Jang-Yen Wu and Howard Prentice

Title: TREATMENT FOR ISCHEMIC STROKE; U.S. Patent Number: 9827220 Date issued: 11/28/2017; Inventors: Jang-Yen Wu and Howard Prentice

Title: TREATMENT FOR ISCHEMIC STROKE; U.S. Patent Number: 10272063 Date issued: 04/30/2019; Inventors: Jang-Yen Wu and Howard Prentice

James and Esther King Biomedical Research Program
Appendix Q
Fiscal Year 2019-2020 Active Grants
Funding Year 2014-2015

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
5JK02	University of Miami	Michael Campos, MD	$1,951,531	$1,844,196.79	$107,334.21	5/25/2015	5/15/2020	No	Yes	Yes
5JK03	H. Lee Moffitt	Vani Nath-Simmons, PhD	$1,904,351	$1,809,133.45	$52.30	5/25/2015	5/15/2020	No	Yes	No

1. **Grant #: 5JK02** Adverse Airway Effects of Inhaled Nicotine from Tobacco and E-Cigarettes

   Principal Investigator: Michael Campos, MD

   Organization: University of Miami

   Grant Progress Report: The main goal of this work is to study if Electronic Cigarette (EC) vapors are toxic to the cells that line up our airways. The research team has been working on experiments using human airway cells grown in the laboratory in special plates and exposing them to different types of electronic cigarette vapors. The effects of EC were tested in volunteers, by getting samples from the cells in the nose, which are similar to the cells that line up the airways.

   One important finding is that vaping e-liquid without nicotine, as short as seven days, produces significant increases in inflammation (at the gene and protein levels). Vaping also affects some pores in the cells (called channels) that normally regulate the fluid that exist over the cells, important for lung protection (the so called mucociliary clearance system). We discovered how some of these injuries occurs at the molecular level and have expanded the studies to different types of e-liquids. The same vaping effects were observed when measuring cells from the nose of normal volunteers. It was noticed that if the e-liquid consists of pure vegetable glycerol (a common e-liquid), the effect seems to be worse. This was a finding also noted in the airway cells grown in the laboratory. The study also included a small clinical trial in which tobacco smokers were asked to switch completely to EC. Only a small fraction (<20%) was able to do so. The team learned about what vaping habits are associated with vaping success, how the composition of mouth bacteria changes with vaping and how the cells of the nose stay inflamed even if these subjects are not smoking tobacco anymore.

   In conclusion, our studies so far indicate that vaping produces harmful effects to lung cells, comparable to the effects of tobacco cigarettes.

   Follow-on Funding:
   Flight Attendant Medical Research Institute CIA; Novel Anti-inflammatory Therapy for Smoke-Associated CRS; Michael Campos, M.D.; $108,500

   Collaborations:
   Actively collaborating with the following groups of investigators:
   Dr. Santanu Banerjee (University of Miami) to study oral microbiome changes associated with changing tobacco smoking to e-cigarette vaping.

   Drs. Robert Foronjy and Patrick Geraghty (SUNY Downstate, NY) on signaling with tobacco smoke and e-cigarette vaping.

   Mass spec facility at University of Kansas Medical Center (KUMC) for measuring nicotine levels.

   Dr. Marianne Geiser (Institute of Anatomy, University of Bern, Switzerland) on e-cigarette vaping.

   Dr. Nikki Nollen (Dept. of Preventive Health, KUMC) on menthol JUUL vaping.

   Dr. Sunil Abhyankar (Stem Cell Institute, KUMC) using mesenchymal stem cells on e-cigarette vaping and tobacco smoking.

Journals:

Doherty DF, Nath S, Poon J, Foronjy RF, Ohlmeyer M, Dabo AJ, Salathe M, Birrell M, Belvisi M, Baumlin N, Kim MD, Weldon S, Taggart C, Geraghty P. Protein Phosphatase 2A Reduces Cigarette Smoke-induced Cathepsin S and Loss of Lung Function. Am J Respir Crit Care Med. 2019 Jul 1;200(1):51-62. doi: 10.1164/rccm.201808-1518OC. (Published)

Poon J, Campos M, Foronjy RF, Nath S, Gupta G, Railwah C, Dabo AJ, Baumlin N, Salathe M, Geraghty P. Cigarette smoke exposure reduces leukemia inhibitory factor levels during respiratory syncytial viral infection. Int J Chron Obstruct Pulmon Dis. 2019 Jun 18; 14:1305-1315. doi: 10.2147/COPD.S196658. eCollection 2019. (Published)

Nath S, Ohlmeyer M, Salathe MA, Poon J, Baumlin N, Foronjy RF, Geraghty P. Reply: Relevance of the PP2A Pathway in the Molecular Mechanisms of Chronic Obstructive Pulmonary Disease. Am J Respir Cell Mol Biol. 2019 Nov;61(5):659-660. doi: 10.1165/rcmb.2019-0116LE. (Published)
Chung S, Baumlin N, Dennis JS, Moore R, Salathe SF, Whitney PL, Sabater J, Abraham WM, Kim MD, Salathe M. Electronic Cigarette Vapor with Nicotine Causes Airway Mucociliary Dysfunction Preferentially via TRPA1 Receptors. Am J Respir Crit Care Med. 2019 Nov 1;200(9):1134-1145. doi: 10.1164/rccm.201811-2087OC. (Published)

Garth J, Easter M, Skylar Harris E, Sailland J, Kuenzi L, Chung S, Dennis JS, Baumlin N, Adewale AT, Rowe SM, King G, Faul C, Barnes JW, Salathe M, Krick S. The Effects of the Anti-aging Protein Klotho on Mucociliary Clearance. Front Med (Lausanne). 2020 Jan 24; 6:339. doi: 10.3389/fmed.2019.00339. eCollection 2019. (Published)

Chung S, Bengtson CD, Kim MD, Salathe M. CrossTalk Opposing View: E-cigarettes Expose Users to Adverse Effects of Vapors and the Potential for Nicotine Addiction. The Journal of Physiology, 2020. doi: 10.1113/JP279271. Online ahead of print. (Published)

Chung S, Bengtson CD, Kim MD, Salathe M. CrossTalk: Rebuttal from Samuel Chung, Charles D. Bengtson, Michael D. Kim and Matthias Salathe. The Journal of Physiology, 2020. doi: 10.1113/JP280093. Online ahead of print. (Published)

Kim MD, Baumlin N, Dennis JS, Yoshida M, Kis A, Aguiar C, Schmid A, Mendes E, Salathe M. Losartan Reduces Cigarette Smoke-induced Airway Inflammation and Mucus Hypersecretion. ERJ Open Research. (Submitted)

Campos M., Fernandez M., Wanner A., Holt G., Donna E., Mendes E., Jaric M., Silva-Herzog E., Schneper L., Segal J., Moraga Amador D., Riveros J.D., Aguiar-Pulido V., Cickovski T., Banerjee S., Salathe M., Mathee K., Narasimhan G. Lower respiratory tract microbiome composition and community interactions in smokers. Microbiome (Submitted)

Leni Z, Cassagnes LE, Daellenbach KR, El Haddad I, Vlachou A, Uzu G, Prevot AAH, Jaffrezo JL, Baumlin N, Salathe M, Baltensperger U, Dommen J, Geiser M. Oxidative Stress- induced Inflammation in Susceptible Airways by Anthropogenic Aerosol. PLOS ONE (in revision).(Submitted)

Patents: None at the time of reporting

2. **Grant #: 5JK03** Expanding the Reach of a Validated Smoking-Cessation Intervention: A Spanish Language Clinical Trial

   Principal Investigator: Vani Nath Simmons, PhD

   Organization: Moffitt Cancer Center

   Grant Progress Report: Tobacco smoking is the leading preventable cause of cancer mortality. Self-help interventions, like cessation booklets, have much wider potential reach than counseling but lower efficacy, except for the extended self-help smoking intervention developed by the Moffitt Cancer Center research team. Distributing the Stop Smoking for Good cessation booklets over 18 months was cost-effective and efficacious. Significant improvement on the efficacy of self-help interventions with high dissemination potential would have large public health impacts on smoking-related morbidity and mortality. Availability of a validated Spanish-language version would enhance impact by reaching the largest population of minority smokers. Florida's Hispanic smokers have prevalence rates mirroring the national Hispanic average (12.6% vs 12.4%) and face challenges unique to Hispanics nationwide such as lower awareness and acceptance of pharmacotherapies and less cessation assistance from health providers. This study's goal is to expand the reach of an evidence-based, self-help intervention by developing and testing a Spanish-language version. This would represent an easily disseminable, low-cost intervention with significant public health impact for Hispanic smokers in Florida and elsewhere. The project advances several research priorities, including health disparities; prevention and treatment; and tobacco use.

   Develop a culturally appropriate self-help intervention for Spanish-speaking smokers. The validated smoking cessation intervention developed by the research team will be adapted for Spanish-speaking smokers, utilizing a systematic, multi-phase "transcreation" process.
   Test the efficacy of the self-help intervention among Spanish-speaking smokers in a Randomized Controlled Trial (RCT). Participants will be randomized to receive the Spanish-language Stop Smoking for Good (SS-SP) intervention or Usual Care (UC) comprising an existing booklet from the National Cancer Institute. Hypothesis: SS-SP will produce higher abstinence rates than UC through 24 months. In prior reporting periods, Aim 1 was completed resulting in the development of a Spanish- language smoking cessation intervention: a series of ten booklets, nine supportive pamphlets and a family support booklet that addressed unique barriers and issues relevant to Hispanic smokers.

   During the previous reporting period, recruitment for the RCT was completed, with 881 Hispanic smokers screened and 555 participants eligible, enrolled, and returned baseline assessments. Administration of the six and 12-month follow up assessments, as well as the 12-month biochemical verification of smoking abstinence were completed. A paper describing the creation of the self-help materials was published (Journal of Health Communication). During the current reporting period, administration of 18 and 24-month follow-up assessments, and 24-month biochemical verification of smoking abstinence were completed. The completed baseline, 6, 12, and 18-month follow-up data have been coded and preliminary analyses have been conducted. A manuscript describing the design, methods, analysis plan, and baseline characteristics of the RCT, submitted for publication during the previous reporting period, was published. Another paper describing multiple strategies employed to recruit Hispanic smokers into the RCT was submitted and accepted for publication (currently in press). In addition, preliminary findings of the RCT were presented at the Society for Research on Nicotine and Tobacco Annual Meeting in March and the 2020 Moffitt Scientific Symposium in May.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals:
Medina-Ramirez, P., Brandon, T. H., Sutton, S. K., Martinez, U., Meade, C. D., Byrne, M. M., Brandon, K. O., Meltzer, L. R., Gonzales, F. M., Simmons, V. N. A randomized controlled trial of a smoking cessation self-help intervention for Spanish-speaking Hispanic smokers: Study design and baseline characteristics, Contemp Clin Trials, 2019,85: 105836. doi:10.1016/j.cct.2019.105836 PMCID: 31473331

Medina-Ramirez, P., Calixte-Civil, P., Meltzer, L.R., Brandon, K.O., Martinez, U., Sutton, S.K., Meade, C.D., Byrne, M.M., Brandon, T.H., Simmons, V.N. Recruiting Spanish-Preferring preferring Participants for a Smoking Cessation Trial in the United States: Comparison of Methods by Enrollment and Cost, J Med Internet Res., 2020, 0(0):e0.doi:10.2196/19389 URL: http://www.jmir.org/2020/0/e0/

Patents: None at the time of reporting

James and Esther King Biomedical Research Program
Appendix R
Fiscal Year 2019-2020 Completed Grants

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
5JK01	University of Miami	David Lee PhD	$1,953,000	$1,901,623.37	$51,376.63	5/25/2015	11/15/2019	No	No	No

1. **Grant #:** 5JK01 Addressing Tobacco Health Disparities via Group Intervention

   Principal Investigator: David J. Lee, PhD

   Organization: University of Miami

   Grant Progress Report: This is a dual-site Randomized Controlled Trial (RCT) testing the effects of group-based cognitive behavioral therapy (CBT) for smoking cessation, with a particular emphasis on eliminating racial/ethnic cessation disparities. The two sites are the University of Miami (UM) in Miami, FL and the Moffitt Cancer Center (MCC) in Tampa, FL. This trial encountered two main challenges, recruitment of Hispanic participants and long-term retention. Our goal was to recruit equal proportions of African Americans, Hispanics, and non-Hispanic Whites. It was anticipated at the start of the study that MCC would recruit the larger population of Whites (an estimated 70% non-Hispanic Whites and 30% minority participants), while UM would recruit a majority of African Americans and Hispanics. We had difficulty reaching the accruals for Hispanic and non-Hispanic White smokers. To address that challenge, we reallocated resources to allow MCC to continue their successful strategies to recruit non-Hispanic Whites, while UM made a protocol amendment to deliver the interventions in Spanish, increasing access for Spanish monolingual smokers. These strategies helped us to accomplish our goal, however, they had an impact over our study timeline. To complete all the follow-up assessments with the remaining cohort groups we applied for a no-cost extension to our project period. This extension allowed us to complete all the assessments with the group cohorts that initiated the intervention during the last year and were mostly Hispanic. The last 12-month follow up was completed on November 2019.

   Follow-on Funding: None at the time of reporting

   Collaborations:

   Journals:
   Reasons for exclusion from a smoking cessation trial: An analysis by race/ethnicity, Monica Webb Hooper, Taghrid Asfar, Asha Dorsey, John B. Correa, Marina Unrod, Karen, Brandon, Vani N., Simmons, Michael H., Antoni, David J. Lee, & Thomas H. Brandon. Ethnicity & Disease. January 2019.

   Patents: None at the time of reporting

Live Like Bella Pediatric Cancer Research Initiative
Appendix S
Fiscal Year 2019-2020 Newly Awarded Active Grants

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
20L01	Florida State University	Akash Gunjan, PhD	$219,138	$0.00	$219,138.00	6/11/20	5/31/23	No	No	No
20L02	Moffitt Cancer Center	Damon R. Reed, MD	$787,272	$32,803	$754,469	4/22/20	4/30/24	No	No	No
20L03	Moffitt Cancer Center	Jianguo Tao, MD, PhD	$219,138	$12,175	$206,963	4/27/20	4/30/23	No	No	No
20L04	Nemours Children's Hospital	Tamarah Westmoreland, MD, PhD	$219,138	$12,175	$206,963	5/7/20	4/30/23	No	No	No
20L05	University of Central Florida	Cristina Fernandez-Valle, PhD	$218,572	$0.00	$218,572.00	6/10/20	5/31/23	No	No	No
20L06	University of Central Florida	Li-Mei Chen, MD, PhD	$109,569	$0.00	$109,569.00	6/10/20	5/31/23	No	No	No
20L07	University of Florida	Elias J. Sayour, MD, PhD	$788,897	$0.00	$788,897.00	6/11/20	5/31/23	No	No	No
20L08	University of Florida	Coy Heldermon, MD, PhD	$219,138	$0.00	$219,138.00	6/12/20	5/31/23	No	No	No
20L09	University of Miami	Julio Barredo, MD	$219,138	$0.00	$219,138.00	6/11/20	5/31/22	No	No	No

1. **Grant #** 20L01 Targeting wild-type Isocitrate Dehydrogenase (IDH) enzymes for treating lethal pediatric Diffuse Intrinsic Pontine Gliomas (DIPG) driven by histone H3.3 K27M mutations

   Principal Investigator: Akash Gunjan, PhD

   Organization: Florida State University College of Medicine

   Abstract of Proposed Research: DNA is our genetic material and it regulates to all aspects of human health, including diseases such as cancer. Histones are small, positively charged nuclear proteins that bind DNA and package it into chromosomes, thereby regulating all DNA associated functions. Isocitrate dehydrogenases (IDH) are important cytoplasmic metabolic enzymes involved in converting isocitrate to a-ketoglutarate (a-KG) during the Kreb's citric acid cycle to subsequently provide both energy and building blocks for the cells. Glioblastomas are brain tumors that affect individuals of all ages, often leading to death. In children, mutations in histone H3.3 are known to drive high grade glioblastomas, including ~80% of all cases of the incurable "Diffuse Intrinsic Pontine Gliomas" (DIPG) that are associated specifically with the H3.3 K27M mutation. On the other hand, a majority of glioblastomas in adults have been linked to mutations in IDH1 and IDH2 enzymes. This has led to the suggestion that childhood and adult glioblastomas may result from defects in very different cellular pathways. However, we have recently obtained preliminary data that point to intriguing molecular connections between histones and IDH enzymes. These data may shed new light on the development of glioblastomas in children and adults and suggest that such tumors may arise via defects in similar cellular pathways in both the age groups. The research team hypothesize that histone binding to IDH enzymes enhances their activity and leads to higher levels of a-KG, which in turn drives excessive histone and DNA demethylation via the a-KG dependent dioxygenases. The resulting DNA and histone hypomethylation is readily observed in H3.3 K27M mutant cancer cells and is likely to drive carcinogenesis via inappropriate expression of genes. Hence, by inhibiting IDH enzymes, it may be possible to ameliorate the defects associated with DNA and histone hypomethylation in H3.3 K27M mutant cancer cells, possibly with therapeutic benefits.

   Based on this idea, the team has developed a potential therapeutic strategy for eliminating H3.3 K27M mutant tumor cells in culture using a novel IDH1 inhibitor to boost their abnormally low levels of histone and DNA methylation prior to radiation therapy. Here, the team proposes to use pediatric patient derived glioblastoma cells carrying either the wild type or mutant H3.3 to define the role of histone binding to IDH enzymes and test the effectiveness of IDH inhibitors in eliminating human H3.3 K27M mutant tumors engrafted in mice. Our Specific Aims are:

   Determine the functional consequences of histone H3.3 binding to IDH1 in the presence and absence of IDH1 inhibition using biochemical, cell biological and genomic assays.

   Measure the effects of IDH1 inhibition with or without concomitant radiotherapy on the growth of human H3.3K27M mutant tumors in a mouse xenograft model.

   The studies proposed here will define the functional consequences of the interaction of histone H3.3 with IDH enzymes. This will provide molecular links between the genes that are mutated in adult and pediatric glioblastomas and enable a better understanding of the overall contribution of H3.3 in cancer prevention. More importantly, the proposed studies will evaluate the potential of IDH inhibitors in therapeutic strategies for H3.3 K27M mutant cancers that are currently incurable. If successful, our studies can quickly lead to highly targeted therapeutic strategies for H3.3 K27M mutant tumors.

   Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

2. **Grant #:** 20L02 Evolutionary Inspired Therapy for Newly Diagnosed, Metastatic, Fusion Positive Rhabdomyosarcoma

Principal Investigator: Damon R. Reed, MD

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

Abstract of Proposed Research: Metastatic Fusion Positive (alveolar) Rhabdomyosarcoma (FPRMS) has a very poor prognosis with only 6% of patients on the most recent Children's Oncology Group trial still alive and without progression of their disease three years after being diagnosed. Even fewer are cured. Treatment for these patients includes intensive chemotherapy timed every three weeks and any of eight agents have been shown to be equally effective with most clinicians treating with the same three drugs as in 1977. The genetic changes that underlie FPRMS have been known since the mid 1990's, and the search for a magic bullet to target this genetic change has not been successful.

Throughout the vast majority of the country, including Florida, there have not been any clinical trials over the past few years for these high-risk patients at the time of their diagnosis. The team believes fundamentally different approaches are needed towards improving therapy for high risk FPRMS. The patients and families need options. The research team has been thinking for a long time that cancers are more like an adaptable invasive species than an infection and this means a more complex elimination strategy is needed than a single good antibiotic drug. Like citrus greening, which remains an unsolved problem despite industry, academic and policymakers all united economically towards its elimination. This analogy highlights the challenge of eradicating a cancer like FPRMS. Nature has eliminated species millions of times through extinction, and it is our belief there are lessons within the understanding of extinction that can help FPRMS patients. With this funding, a trial is being conducted with 3 novel treatment options, in addition to a standard of care option, for newly diagnosed FPRMS patients and their physicians to choose from. An enhanced first strike that combines the best agents for newly diagnosed and relapsed patients. A second strike or maintenance option that changes therapy when a patient has maximized benefit from the first intervention, again using the most effective agent at relapse, and an adaptive strike that manages rather than eliminates FPRMS for a longer period of time

All three options have the same shared goal of significantly improving the disease control rate from 6% to 35%, three years after diagnosis. The trial adds conceptually to the armamentarium of the oncologist, patient and family as well and so will hopefully have implications beyond this subset of patients.

This concept has been co-created by an extensive and diverse team of scientists and clinicians and reviewed by families who lost a child to FPRMS. Standard (scans) and novel disease evaluation tools such as blood tests to detect markers of FPRMS and mathematical models of disease resistance will also be studied as part of this trial. Substantial progress has been made towards opening this trial at Moffitt and across Florida sites thanks to this grant. The infrastructure has been fully developed to safely conduct this novel trial over the first 3 months of funding.

Follow-on Funding: None at the time of reporting

Collaborations:
Investigators at sites throughout Florida in the existing Sunshine Project consortium can open this trial and have been made aware that the trial is available. Progress has been made at all sites towards opening this trial in these early days. More specifically this study will be activated by the end of July at Moffitt Cancer Center:

Pending Activation Florida Sites:

University of Miami: working on budget and contracts with Moffitt team Nemours Jacksonville: working on budget and contracts with Moffitt team Nemours Orlando: working on budget and contracts with Moffitt team

UF: working on budget and contracts with Moffitt team

JHACH: working on budget and contracts with Moffitt team and soon to submit to the local IRB

Journals: None at the time of reporting

Patents: None at the time of reporting

3. **Grant #:** 20L03 New Therapeutic Vulnerabilities for Pediatric Burkitt Lymphoma

Principal Investigator: Jianguo Tao, MD, PhD

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

Abstract of Proposed Research: Pediatric Burkitt Lymphoma (PEBL) is the most common (about 40%) non-Hodgkin lymphoma in children and adolescents in western countries. The prognosis of PEBL has steadily improved over the past 30 years through the introduction of intensive chemotherapeutic regimens with 80% 5-year overall survival. However, this success has come at the cost of the significant chemotherapy-induced acute toxicity secondary to intensive chemotherapy, requiring the need to identify less toxic but targeted therapy. Furthermore, approximately 20% of patients with PEBL refractory or relapsed, developed chemotherapy-resistant disease and these patients can rarely be salvaged. These results suggest the urgent need to identify alternative approaches focusing on targeted therapy to circumvent chemotherapy-toxicity and resistance by providing alternative or more precise therapeutic targets. Here, we tested our hypothesis that Myc and MCL-1 cooperatively drive and sustain PEBL, and MCL-1 is a major determinant that governs PEBL response and resistance to chemotherapy. The research team proposes the following progress to determine the role of MCL-1 protein in PEBL survival and drug response.

The team has evaluated the interplay of Myc and MCL-1 in the maintenance of PEdiatric Burkitt Lymphoma (PEBL) survival and growth. It was observed that Myc down-regulation decreased MCl-1 expression but have no effect on other anti-apoptotic proteins BCL-2. Blocking MCL-1 with MCL-1 specific inhibitor or gRNAs against MCL-1 induced significant PEBL line cell apoptosis. Together, these results support the key role of MCL-1 in PEBL cell growth and survival.

To determine the mechanisms of resistance to MCL-1 inhibitor, S63845. The team determined the role of JAK-STAT-BCL-XL pathway in the development of MCL-1 inhibitor (S63845) resistance in PEBL cells. The team designed and is employing a panel of specific gRNAs using CRISPR/cas9 editing to knockdown (KD) STAT1 and STAT3 to examine the impact of these KDs on cell viability, colony

formation potential and drug response. From our initial preliminary experiments, it seems that S63845 resistant BL lines are sensitive to STAT1/3 knockdown. The underlying mechanism and pathways for the STAT1 and STAT-3 impact on cell viability and clonogenic growth of S63845 resistant clones will be determined.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

4. **Grant #: 20L04** Zika Virus Mediated Lysis of CD24 Positive Neuroblastoma

Principal Investigator: Tamarah J. Westmoreland, MD, PhD

Organization: Nemours Children's Hospital

Grant Progress Report: Progress in the grant specific aims has been excellent with focus on Aim 1, which is to create the Cisplatin resistant neuroblastoma cell lines. The laboratory at the University of Central FL was allowed to resume research in the latter part of May 2020 because of the pandemic. Research staff have grown the grant cell lines and are in the process of incorporating a luminescent marker that will be important for the Aim 2 mouse research. Staff have also conducted Cisplatin dose studies to confirm the correct dose to create the Cisplatin resistant cell lines. Staff soon will be ready to expose the neuroblastoma cell lines to the Cisplatin to create the lines for Aim 1.

Follow-on Funding: None at the time of reporting

Collaborations:
Collaboration with the University of Central Florida is key to the success of this grant. Staff collaborate with Dr. Griffith Parks in the Burnett School of Biomedical Sciences. Dr. Parks serves as a collaborator on the project as well as the supplier of the Zika virus.

Journals: None at the time of reporting

Patents: None at the time of reporting

5. **Grant # 20L05** Development of an Early Diagnostic Test for Malignant Tumors in Children with NF1

Principal Investigator: Cristina Fernandez-Valle, PhD

Organization: University of Central Florida

Abstract of Proposed Research: Neurofibromatosis Type 1 (NF1) is a common and complex tumor disorder affecting one in 3,000 individuals. It is caused by mutations in the neurofibromin tumor suppressor gene. Children born with NF1 develop benign tumors called neurofibromas that grow on or below the skin as well as along any nerve inside the body. The neurofibromas can grow very large and disfigure the face, body and skeleton. One type of tumor, a plexiform neurofibroma, grows within the

body and often cannot be removed surgically. These tumors have a high risk of becoming a Malignant Peripheral Nerve Sheath Tumor (MPNST). There is no effective treatment for a MPNST as surgery and chemotherapy are ineffective. Development of an MPNST is the leading cause of death in NF1 patients who succumb within one to two years of diagnosis. NF1 children with plexiform neurofibromas are followed clinically on a yearly basis but there is no diagnostic test to determine if a tumor has become malignant until the patient presents with unusual pain. By then the tumors are large and prognosis is very poor. The objective of this study is to develop a diagnostic non-invasive blood test that can be incorporated into the yearly exam of NF1 children that will detect the presence of circulating tumor cells. The method to be developed is based on the use of the CELLSEARCH instrument. It is approved by the Food and Drug Administration for use in patients with metastatic breast, colorectal, or prostate cancer. We will adapt and optimize the current established protocols for detection of MPNST with the long-term goal of translating the method into the clinic to aid in management of NF1 in children. Early detection of MPNST should lead to more successful treatment of MPNST and provide peace of mind if the result is negative.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

6. **Grant #:** 20L06 Exosome-Mediated Activation of Matriptase Targeting B-Cell Lymphoma

Principal Investigator: Li-Mei Chen, MD, PhD

Organization: University of Central Florida College of Medicine

Abstract of Proposed Research: Lymphoma is the third most common childhood malignancy that originates in the lymph system. It affects children in age of 10-20. Each year in the US, there are about 800 cases of the non-Hodgkin's (NHL) Lymphoma, more commonly reported in children. Current treatments for NHL are mostly chemotherapy and surgery (for stage I and II only). Although the five-year survival rate can reach more than 80%, the prognosis is very poor in refractory patients. Recent research on B-cell lymphoma indicated that a type-II membrane serine protease matriptase is over-expressed in some B-cell lymphoma cell lines as well as in lymphoma patient specimens. Over-expressed matriptase in these cells promote cancer cells' invasive potentials. The matriptase protein is mostly expressed in epithelial cells and its activity is greatly regulated by its cognate inhibitors, e.g., HAI-1 or HAI-2 (hepatocyte growth factor activator inhibitor-1 or-2). Reduced expression of these inhibitors will unleash matriptase activity by an auto-activation pathway. Uncontrolled active matriptase is detrimental to epithelial cell integrity resulting in premature placenta development which is lethal to embryos, or unprotected skin formation causing neonatal death after birth, or cellular apoptosis. Activation of matriptase can be achieved by another membrane-bound serine protease prostasin. We have previously shown that over-expression of recombinant prostasin in matriptase-expressing cells reduced the level of full-length matriptase in those cells and generated the serine protease domain, which could subsequently activate additional matriptase and perpetuate the auto-activation pathway. Other studies have shown that matriptase expression down-regulation or silencing in B-cell lymphoma cell lines reduced invasiveness.

Exosomes are small membrane vesicles produced and released by most eukaryotic cells. They are

generally below 100 nm in diameter but contain specific sets of membranous and cellular proteins and nucleic acids. Exosomes are capable of merging with other cells via membrane fusion and deliver the exosome cargos to other cells. Taking advantage of these exosome characteristics, exosome-based therapy has become one of the most studied delivery vehicles in recent years. In this application, for proof of concept, we intend to use exosome-based delivery of prostasin to B-cell lymphoma cell lines for activation of matriptase in B-cells. The innovation on prostasin-mediated matriptase activation to reduce the total content of matriptase in B-cells is that once activated, matriptase could go on to the auto-activation pathway in a perpetual manner, which eliminates the need of repeated delivery, as in the case of matriptase-targeting siRNAs for down-regulating matriptase expression. The exosome-treated B-cells will be subjected to proliferation and invasion experiments. The goal of this project is to develop an exosome-based treatment method for a future preclinical study with a Patient-Derived Xenograft model. If successful, it can be used as an alternative treatment for B-cell lymphoma, especially for relapse therapy, as those patients may not be suitable for a second round of chemotherapy.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

7. Grant # 20L07 Multi-Center Phase I Study Evaluating Lipid-Nanoparticle Vaccines Against Pediatric High-Grade Glioma

Principal Investigator: Elias Sayour, MD, PhD

Organization: University of Florida

Abstract of Proposed Research: Primary High-Grade Gliomas (pHGGs) in children remain almost uniformly lethal, and thus necessitate development of novel targeted therapeutics. Our group has developed a novel treatment platform, which leverages the use of clinically translatable nanoparticles (NPs) combined with tumor derived mRNA that simultaneously functions as both a vaccine and an immunomodulating agent. Our team has previously translated mRNA loaded dendritic cell (DC) vaccines for patients with glioblastoma (NCT0246528_PI: Mitchell) and pediatric patients with pHGGs (NCT03334305_PI: Sayour) and DIPG (NCT03396575_PI: Sri Gururangan). Despite promising preclinical data (Mitchell et al. *Nature* 2015), DC vaccines remain mired by significant cost, complexity and time to generation. RNA-NPs are expected to be superior based on their m anufacturing/commercialization properties and capacity for enhancement. To facilitate translation of this promising technology into first-in-human studies for pHGG, the team has partnered with the Pacific Pediatric Neuro-Oncology Consortium (PNOC), where our clinical trial concept has been accepted for multi- institutional trial development. After a successful pre-IND meeting, the team is now in the final stages of compiling our final FDA-IND package for submission (Summer 2019). Our team anticipates being in position to conduct a multi-institutional trial evaluating the safety, immunogenicity and anti-tumor efficacy of tumor mRNA-NPs in pediatric patients with pHGG (by March of 2020).

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

8. **Grant #:** 20L08 Novel Immunologic Therapy of Soft Tissue Sarcoma

   Principal Investigator: Coy Heldermon, MD, PhD

   Organization: University of Florida

   Abstract of Proposed Research: Sarcomas are soft tissue cancers that are more common in children than adults. Mutation in the p53 gene results in a 40% chance of developing cancer before age 20, with the most common tumor type being sarcomas. Therapy of sarcoma is predominantly surgical with modest effectiveness of prolonged chemotherapy on survival in metastatic disease. Dr. Heldermon will use an immune competent mouse model with p53 deficiency that develops sarcomas at a high rate to determine the effectiveness of Dr. Sayour's lipid-nanoparticle technology for delivery of tumor derived RNA. This therapy harnesses the immune system to combat the tumor by increasing immune recognition of the mutated genes specific to the tumor. Together these PIs will determine the effectiveness of the tumor derived RNA lipid-nanoparticles to mount this immune response and treat sarcoma.

   Follow-on Funding: None at the time of reporting

   Collaborations: None at the time of reporting

   Journals: None at the time of reporting

   Patents: None at the time of reporting

9. **Grant #:** 20L09 Targeting Compensatory Survival Responses at the Intersection of Energy Metabolism and Epigenetics in Acute Lymphoblastic Leukemia

   Principal Investigator: Julio Barredo, MD

   Organization: University of Miami

   Abstract of Proposed Research: Despite recent advances in immunotherapy and targeted therapy for Acute Lymphoblastic Leukemia (ALL), relapsed/refractory ALL continues to be the most common cause of cancer related death in children and adolescents. In addition, these modalities exhibit significant limitations. In the case of targeted therapies, the heterogeneity of ALL precludes efficacy across ALL sub-types, and several novel mechanisms of resistance are emerging for immunotherapy approaches. The research staff hypothesize that targeting fundamental processes that ALL cells use for growth and survival will overcome some of the above limitations and lead to more widely applicable treatment strategies. The team had previously discovered that blocking the mechanisms that ALL cells use to adapt to energy and metabolic stress is a highly effective therapeutic strategy. Our previous work has also shown that a protein called AMP activated protein kinase (AMPK) is critical for cells to adapt to low energy and metabolic stress. The regulation of cancer genes is dependent on the conformation of DNA,

structurally called chromatin and its regulation referred as epigenetics. Moreover, metabolism has emerged as a central component of the epigenetic control of cancer genes. Our recent work has shown that AMPK binds to specific areas on chromatin leading to changes in gene expression, and that this binding changes in response to energy and metabolic stress. The transcription factors NELFE, TAF1 and Integrator (INT) are among those we identified that interact with AMPK on chromatin, and this interaction is modified in response to energy/metabolic stress in ALL cells leading to changes in gene expression. The fundamental goal of this proposal is to elucidate the mechanism(s) of epigenetic control of energy/metabolic stress and adaptive and survival responses in ALL regulated by AMPK and exploit our findings to develop unique opportunities for epigenetic-based therapeutic interventions. To accomplish this goal, the team will first elucidate how AMPK induces epigenetic changes in gene expression in ALL cells as an adaptive survival response to conditions of energy/metabolic stress. Second, determine the partners of AMPK forming the putative regulatory complex attached to chromatin we have identified that lead to changes in gene expression to allow ALL cells to survive these conditions of stress. These experiments will allow us to identify genes, transcription factors, and chromatin-associated proteins in the epigenetic networks that ALL cells use to survive energy/metabolic stress that can be targeted for therapeutic gain. Hence, we anticipate this strategy will potentially benefit a broad range of ALL patients. Findings from this proposal will identify novel targets that will synergize with existing therapies or other genetic strategies to help us design new treatments and increase cure rates for children and adolescents with relapsed/refractory ALL. Staff believe this novel approach will overcome resistance/refractoriness to existing therapies and will have wide applicability to most sub-types of ALL.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

Live Like Bella Pediatric Cancer Research Initiative
Appendix T
Fiscal Year 2019-2020 Active Grants
Funding Fiscal Year 2018-2019

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
9LA01	Florida State University	Q.X. Amy Sang, PhD	$250,000	$111,104.00	$138,896	4/17/2019	3/31/2022	No	No	No
9LA02	H. Lee Moffitt Cancer Center	Mihaela Druta, MD	$784,733	$266,568	$523,165	3/29/2019	3/31/2023	No	No	No
9LA03	H. Lee Moffitt Cancer Center	Keiran Smalley, PhD	$250,000	$111,104	$138,896	4/02/2019	3/31/2022	No	No	No
9LA04	University of Florida	Jatinder Lamba, PhD	$223,758	$62,155	$161,603	4/05/2019	3/31/2022	No	No	No
9LA05	University of Florida	Zhijian Qian, PhD	$250,000	$111,104	$138,896	4/11/2019	3/31/2022	No	No	No
9LA06	University of Miami	Claudia Rodrigues ,PhD	$250,000	$111,104	$138,896	4/03/2019	3/31/2022	No	No	No
9LA07	University of Miami	David Robbins, PhD	$250,000	$97,216	$152,784	4/29/2019	4/30/2022	No	No	No
9LA08	University of Miami	Alan Pollack, PhD, MD	$250,000	$111,104	$138,896	4/01/2019	3/31/2022	No	No	No
9LA09	University of Miami	Julio Baredo, MD Sulangna Banerjee, PhD	$241,509	$107,328	$134,181	4/08/2019	3/31/2022	No	No	No
9LA10	University of South Florida	Mildred Acevedo-Duncan, PhD	$250,000	$91,661	$158,339	5/21/2019	4/30/2022	No	No	No

1. **Grant #:** 9LA01 Engineering Human Childhood Brain Malignant Rhabdoid Tumor Organoids

   Principal Investigator: Q.X. Amy Sang, PhD

   Organization: Florida State University

   Grant Progress Report: Brain and other central nervous system cancer are one of the most common types of cancer in children. Atypical Teratoid Rhabdoid Tumor (ATRT) is a rare and very aggressive type of human pediatric brain cancer that mostly arises from the cerebellum located at the hindbrain region. A human cerebellum brain organoid model has been built by our team using induced Pluripotent Stem Cell line (iPSC). This proposed project is building a novel 3-dimensional spheroid model that mimics human pediatric brain rhabdoid tumor formation. The state-of-art CRISPR-Cas9 gene editing and stem cell technologies are utilized to generate this novel human pediatric brain cancer model for future drug evaluation and development for the effective treatment of pediatric brain cancer patients. The central hypotheses are that human pediatric brain malignant rhabdoid tumor is originated from early Neural Progenitor Cells (NPCs) after the inactivation of the SMARCB1 tumor suppressor; thus, deleting the SMARCB1 gene in early NPCs may generate a rhabdoid tumor model for therapeutic evaluation. ATRT mostly arises from the cerebellum located at the hindbrain region. Thus, a human cerebellum brain organoid model is built using commercially available induced pluripotent stem cell lines (iPSK3 and EpiPSC). ATRT is characterized by the biallelic inactivation of a tumor suppressor gene SMARCB1 and has high embryonic gene expression profile. Thus, guide RNA molecules are designed and CRISPR-Cas9 gene-editing technology is used to knock out the SMARCB1 gene to mimic human ATRT development in childhood. ATRT is characterized by the biallelic inactivation of a tumor suppressor gene SMARCB1 and has a high embryonic gene expression profile. The guide RNA molecules have been designed, and CRISPR-Cas9 gene-editing technology has been used to knock out the SMARCB1 gene to mimic human ATRT development in childhood. The gene knock-out construct was transfected into induced pluripotent stem cells and experiments were performed to verify if the SMARCB1 gene is knocked out. DNA sequencing experiments are carried out to verify the gene knock out, and Western (immune) blot experiments are carried out to further verify the SMARCB1 protein is not produced by the stem cells or the neural spheres after the gene knockout experiments are carried out. Our preliminary results indicated that we have generated mutations in SMARCB1 gene, and the gene is partially knocked down. We are performing additional experiments to completely knock out or knock down the SMARCB1 gene and build a pediatric brain cancer model for future therapeutic testing. Treatments for ATRT are ineffective due to the lack of understanding the molecular mechanisms and effective pre-clinical investigative models for drug testing. The treated patients have a high recurrent rate with more aggressive cancer phenotype. Current treatments for pediatric cancer patients have a significant long-term side effect on children’s growth and development. The drug effect can be different to a child than to the adult. Thus, this research may lead to a novel and powerful experimental ATRT organoid model to be used for drug screening, evaluation, and testing.

   Follow-on Funding: None at the time of reporting

   Collaborations: None at the time of reporting

   Journals: None at the time of reporting

   Patents: None at the time of reporting

2. **Grant #:** 9LA02 A Phase Ib/II Study to Evaluate the Safety, Feasibility and Efficacy of Nivolumab or Nivolumab in Combination with Azacitidine in Patients with Recurrent, Resectable Osteosarcoma

   Principal Investigator: Mihaela Druta, MD

   Organization: H. Lee Moffitt Cancer Center and Research Institute,

   Grant Progress Report: The purpose of the study is to see if Nivolumab (Dose Level 1) or Nivolumab in combination with Azacitidine (Dose Levels 2 and 3) given to patients before and after surgery is safe and to see if patients are able to successfully complete the treatment before their surgery without any extended delays in treatment.

   As of October 1, 2019, the research team has had a total of six patients accrued for this study. This is the required number of patients needed to complete the accrual goal for Dose Level 1. A total of seven sites have been activated and are open to enrollment (three sites in Florida and four sites outside of Florida - an additional seven sites are pending activation). Out of the six patients enrolled, three have been removed from therapy due to disease progression and the remaining three are still active and doing well. There have been no serious adverse events caused by being on this study. In addition, none of the patients, so far, have experienced a dose limiting toxicity (DLT) during the DLT time period nor were there any delays for surgery. Prior to proceeding to Dose Level 2, an interim analysis of Dose Level 1 is required to be submitted to the Protocol Monitoring Committee for review. The submission took place July 8, 2020. Upon approval, Dose Level 2 will be open to enrollment. There are quarterly meetings with our Clinical Trials Oversight Committee. The last meeting to take place was on July 13, 2020 where it was decided for the study to continue as designed (doctors not related to the Sunshine Trials review our trials and let us know if there any red flags).

   Follow-on Funding: None at the time of reporting

   Collaborations: None at the time of reporting

   Journals: None at the time of reporting

   Patents: None at the time of reporting

3. **Grant #:** 9LA03 Defining and Modeling Pediatric Melanoma Development

   Principal Investigator: Keiran Smalley, PhD

   Organization: Moffitt Cancer Center

   Grant Progress Report: Melanoma is the deadliest form of skin cancer. Although most commonly considered to be an adult disease, melanoma can also occur in pediatric patients. The incidence of pediatric melanoma is rising, particularly in individuals between the ages of 15-19. Moffitt Cancer Center has developed an integrated program for pediatric melanoma management with one of the largest patient populations in the world. The majority of pediatric melanomas are sporadic and relatively little is known about their molecular basis or the precise etiologic mechanisms. Although there is evidence that neonates and young children have less developed immune systems and may also be uniquely susceptible to the mutagenic effects of Ultraviolet Radiation (UVR), the interplay of these two risk factors in melanoma development is

not known. In the first year of this work the research team has begun to explore the differences in immune responses to UVR irradiation in young and old mice, and then in turn, how this influenced later tumor development. We have made significant progress in understanding the age-related nature of the immune response to UVR. It was found that younger mice had a much-impaired immune response when UV-irradiated compared to the older mice, this was observed in both the lymphocyte and myeloid cell compartments. Samples of UVR treated mouse skin are being collected to perform a detailed characterization and curation of the immune cell infiltrate using a newly developed single cell RNA-Seq platform our group has developed. Over this past year we have made significant progress in developing tools and methods to curate mouse immune cell populations from our data. In our second series of studies, cohorts of mice exposed to UVR at both neonatal and adult ages, in whom a potent oncogene (BRAF V600E) was induced at day 22, for the development of melanomas are being followed. One mouse has developed a melanoma, which was harvested and processed for histopathology, multiplexed immunohistochemistry for immune cell subsets, and single cell RNA-Seq (scRNAseq), and the peripheral blood was analyzed by flow cytometry. An analysis of this first sample showed differences in T-cell activation in tumors compared to peripheral blood relative to the T-cells in the periphery, i.e. in the blood, the T-cells that infiltrated the tumor tissue displayed much lower expression of CD62L, and almost all were CD69+, suggesting they are highly activated compared to their counterparts in the blood. Further evaluation also suggested that these cells retained properties of effector T-cells based on their expression of the effector molecule granzyme B. Lastly, the majority of these tumor-associated T-cells displayed much higher levels of CD103, a protein that is associated with tissue-resident memory T-cells. As these first experiments were successful, the team is planning to expand our experiment to follow larger numbers of mice to determine the effects of UVR upon melanoma development. These studies can determine the effects that the age of sun exposure has upon melanoma development, allowing new prevention and treatment strategies to be developed.

Follow-on Funding: None at the time of reporting

Collaborations:

This project is a collaboration between 5 investigators at Moffitt. These include Cancer Biologist Dr. Keiran Smalley, Pathologist Dr. Jane Messina, Immunologist Dr. Dennis Adeegbe, Mouse Modeler Dr. Florian Karreth and Dermatologist Dr. Kenneth Tsai.

Journals: None at the time of reporting

Patents: None at the time of reporting

4. **Grant #:** 9LA04 Pharmacogenomics and Toxicities of Thiotepa, Busulfan and Fludarabine in Pediatric HSCT Recipients

Principal Investigator: Lamba Jatinder, PhD

Organization: University of Florida

Grant Progress Report: The three main investigators have held regular in person and video-conference monthly meetings in order to discuss implementation of the experimental plan, data acquisition and patient recruitment. Genotyping of 81 single nucleotide polymorphism (SNPs) in patient specimens thiotepa (n=31); busulfan (n=59) and Fludarabine (n=79), samples has been completed. All the identified to be of functional relevance. Assay designs for the list of SNPs

have been finalized. Genotype data has been QCed, missing or failed genotypes has been repeated. The data has been evaluated for minor allele frequency for each SNP and compared with the database of healthy population. Checked for Hardy Weinberg equilibrium. Data has been shared with Dr. Long-Boyle for merging pharmacokinetic data and clinical endpoints. Dr. Long-Boyle has completed running Population PK models for each of the drugs to describe drug clearance and exposure. We have completed the pharmacokinetic model development phase of the work. The goals of this work is to develop a combined population PK model for thioTEPA and its active metabolite, TEPA and to identify sources of variability that contribute to patient disposition including drug clearance and exposure.

Plasma time-concentration data for thioTEPA and TEPA were collected in 14 pediatric patients. Multiple dosing strategies were evaluated depending on diagnosis and included 300 mg/m2, 5 mg/kg or 10 mg/kg administered by a continuous IV infusion over one to two hours. Following a single dose of thiotepa a total of four blood samples were collected were collected as determined through an optimal sampling strategy. The FOCEI maximum likelihood approximation estimation method was used for the model-building process to estimate PK parameters, along with estimation of between-subject variability, and residual unexplained variability. Patient- specific covariates, including gender, age, actual body weight and laboratory markers for hepatic and renal function, were evaluated to quantify the contribution to variability in PK parameters.

A two-compartment Pharmacokinetic (PK) model for both thioTEPA and TEPA with proportional error model best described the data. An empirical allometric scaling of clearance and volume parameters significantly improved the model fit (delta OFV = 8.964, $p<0.05$). The population mean volume of distribution and clearance of thioTEPA from the final population PK model are 12.6 L and 2.5 L/h, respectively, and were in good agreement with those previously reported by TEPADINA prescribing information. Actual body weight was the only significant independent predictor for thioTEPA and TEPA clearance, evaluated thus far. The next steps are to include genotype information in the covariate analysis to better explain between-subject variability in the model.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

5. Grant #: 9LA05 Molecular Basis and Treatment of Pediatric AML with Hyperexpression of EVI1

Principal Investigator: Zhijian Qian, PhD

Organization: University of Florida

Grant Progress Report: This study aims to get a better understanding of the biology of a subset of Pediatric Acute Myeloid Leukemia (AML), which is one of the most common and fatal forms of hematologic malignancies. EVI1 high expression was detected in 10-25% of pediatric and young adult AML with an adverse outcome in these patients. No targeted or individualized therapies on this subset of AML patients are available. Significant progress has been made on

this project during this period. Our study focused on determining how EVI1 high expression contributes to the development of MDS/AML using our newly established animal model, in which the EVI1 gene can be induced to mimic the upregulation of EVI1 gene expression in Hematopoietic Stem/Progenitor Cells (HSPCs) from MDS/AML patients with high EVI1 expression. To understand the molecular mechanisms underlying the role of EVI1 in the development of myeloid malignant diseases, the research team has performed global gene expression HSPCs from both the control and EVI1 transgenic mice. Several interesting downstream targets of EVI1 have been identified. Further, the team has validated the expression of these candidate genes by quantitative Real-Time Quantitative Reverse Transcription polymerase chain reaction (qRT-PCR) in the HSPCs from these mice. Additionally, the effects of upregulation of the candidate gene of proliferation, survival and differentiation of HSPCs from mice has been determined. Of interest, our research found that NGN1 gene overexpression significantly promoted cell survival and proliferation. Currently, construction of a new retroviral vector expressing NGN1 specific shRNAs is underway. In addition, the team also developed in vitro culture system to culture the human HSPCs. By getting better understanding of the molecular mechanism that mediate the function of EVI1 in leukemogenesis, it facilitates identification of new therapeutic strategies for the treatment of children cancers.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

6. **Grant #:** 9LA06 Novel Mechanisms of Anthracycline-Induced Cardiomyopathy

   Principal Investigator: Claudia Rodrigues, PhD

   Organization: University of Miami

   Grant Progress Report: The Rodrigues laboratory at the University of Miami is investigating the mechanisms underlying the toxic effects of anthracyclines, which are highly effective drugs used in the treatment of several types of pediatric cancers. Since their discovery over 50 decades ago, the cardiotoxic effects of anthracyclines remain a significant medical problem. Despite their effectiveness, cancer survivors experience serious chronic and life-threatening effects that can lead to congestive heart failure. The goal of these studies is to identify early mechanisms involved in anthracycline toxicity that can be targeted for the development of cardioprotective therapies. In addition, these studies will be the first to look at age-related mechanisms that contribute to the development of chemotherapy-induced heart failure. During the current award period, the Rodrigues lab investigated the role of a central molecule that regulates a broad range of cellular functions known as c-Myc. For this study, the research group used a genetically modified model in which c-Myc was removed from the cells that line blood vessels, known as endothelial cells. The rationale behind this approach is that endothelial cells are the first ones in direct contact with the chemotherapy agent after administration. As such, they are the first to suffer its impact and can send signals to the organ regulating its function.

The research group has found significant sex-related differences regarding the acute effect of chemotherapy in both control and animals deficient for c-Myc. Control females show an acute impact in cardiac function, while males do not show any significant signs of dysfunction. Interestingly, cardiac function of c-Myc deficient females was not severely impacted, suggesting that c-Myc may have a protective effect. Conversely, although males did not show early signs of cardiac dysfunction, those deficient for c-Myc showed a significant functional impact and signs of cardiac remodeling. These findings indicate that endothelial c-Myc plays a sex-specific role in anthracycline cardiotoxicity. In addition, although echocardiography has been the standard method used for the assessment of cardiac dysfunction as a surrogate of cardiotoxicity in clinic. The findings obtained in this project suggest that this approach must be revised, as it is not clear if this is the best approach to determine future outcome. In the current period, the Rodrigues lab started investigating molecular changes in male and female animals without any genetic modification to better understand the impact of chemotherapy cardiotoxicity and identify mechanisms involved. Similar to the genetic model, initial assessment of males and females indicate significant sex-differences, suggesting that the development of any protective approach must be specific to each sex. This is a longitudinal study that have just started, and the research group expect to provide results that are more definitive in the next two to three cycles.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

7. **Grant #:** 9LA07 Designing New Therapeutic Strategies for the Most Lethal Forms of Medulloblastoma

Principal Investigator: David J. Robbins, PhD

Organization: University of Miami Miller School of Medicine

Grant Progress Report: Brain tumors are the number one cause of cancer related deaths in children, with Medulloblastoma (MB) being the most common. Although the overall five-year survival of MB patients is 70-80%, a significant number of these children respond poorly to standard of care treatment and ultimately succumb to their disease. Based on recent genomic classification efforts, a subset of this latter cohort is characterized by constitutive SONIC HEDGEHOG (SHH) activity and mutations in the TRP53 tumor suppressor gene. Mutations in TRP53 are one of the most common hallmarks of human cancer, loss of which results in significant genomic instability. As a result, large scale alterations in the signaling networks that drive cellular proliferation, differentiation and survival are created, a smaller number of which are subsequently selected for during the tumorigenic process. As directly targeting mutant TRP53 has proven elusive, the research team proposed to identify and target components of signaling networks that regulate TRP53 SHH MB viability. Our preliminary results have identified two distinct drivers of TRP53 SHH MB growth, one of which regulates bulk tumor growth and one of which is required for the maintenance of a small subset of tumor propagating cells. The goal of this proposal is to elucidate the signaling networks regulated by these two MB drivers, identify novel druggable regulators within these networks, and provide pre-clinical proof of concept data that targeting these novel regulators will reduce MB growth. Specifically, we

proposed two Aims:

Identify novel regulators of GLI2 required for TRP53 mutant SHH medulloblastoma viability. The team has shown that one of our prioritized candidates, UHFR1 regulates GLI1 activity in a manner comparable to GLI2 activity. Thus, as UHRF1 is not specific for GLI2, the team will evaluate other candidates from our Gli regulator screens. A list of miR34a targets and used bioinformatics has been identified to arrange these targets into distinct signaling/biological process pathways. Using single cell sequencing and bioinformatics, a list of signaling pathways enriched within a stemness cell cluster in TRP53 mutant SHH MB tissue has also been identified. By comparing these two lists of candidate pathways, the team has identified and prioritized those candidates common to both approaches. Evaluation of the role a subset of these candidates play in MB sphere culture self-renewal ex vivo has begun. Additional hits from our screens this year have been validated. Survival data from a cohort of MB patients showed that higher DNMT1 expression is associated with a worse prognosis. Survival data from a cohort of MB patients showed that higher SMC1A expression is associated with a worse prognosis. A number of proteasome components showed up in our Gli regulator screens screen. Research staff hypothesize that Gli2/Gli3 are likely target of proteasome inhibitors. Identify regulators of tumor propagating cell viability in TRP53 mutant medulloblastoma.

Our preliminary data suggest that the ability of the SOX2+ MB cells to self-renew is regulated by the small non-coding RNA miR34a. In these cells, loss of P53 reduces the expression of miR34a, triggering the activation of a series of miR34a-repressed signaling networks that control tumor propagation, including WNT signaling. A candidate approach centered on miR34a- repressed targets and an approach focused on identifying a stemness enriched cell cluster in SHH MB tissues using single cell RNA sequencing analyses has been carried out. Candidate pathways identified using both approaches were then prioritized based on MB patient outcomes1. The data helped to identify the EPH/EPHRIN signaling pathway as a novel candidate regulator of TRP53 mutant MB self-renewal. EPH receptors constitute the largest family of receptor tyrosine kinases and are activated upon their binding to the EPHRIN ligands. This pathway has been previously linked to brain development and function17, maintenance of the neuronal stem cell niche, and cancer. Our data show that within this pathway, the EPHRIN ligand EPHRIN-B1 is a well-validated miR34a-repressed target that localizes in cells expressing stemness biomarkers or MB propagating cells. Importantly, EPHRIN-B1 gene expression is also enriched in metastatic MB samples and linked to poor outcome in MB patients, specifically those carrying a mutation in TRP53.

This grant cycle started by further validating the role that Ephrin-B1 plays in reducing the self-renewal of TRP53 mutant medulloblastoma cultures. siRNA against Eprhin-B1 was used in 2 independent TRP53 mutant MB cultures. This RNA sequence reduced the expression of the target and the ability of these cells to self-renewal. The team tried to validate our siRNA data on MB self-renewal using two phamacogical inhibitors of EPH/EPHRIN-B1 binding36,39, such as UniPR156 and Urolithin-A. Both compounds were able to reduce the number of secondary spheres in TRP53 mutant MB tissues. Moreover, upon attenuation of Eprhin-B1 the levels of expression of the stemness biomarker SOX2 was reduced. Thus, our data allowed us to identify the EPH/EPHRIN signaling pathway as a novel candidate regulator of TRP53 mutant MB self-renewal. There have been no new experimental advances on this approach but have begun a more extensive bioinformatics analysis of our data to gain further insights.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

8. **Grant #:** 9LA08 Maintaining Renal Function After Total Body Irradiation

   Principal Investigator: Alan Pollack, MD, PhD

   Organization: University of Miami

   Grant Progress Report: The main objectives are to define the sensitivity and mechanisms of kidney damage from radiation, as well as methods to mitigate such damage. As proposed in Aim 1, the research team has determined that single dose bilateral kidney irradiation resulted in a dose-dependent loss of kidney function and fractionated equivalent doses reduced the risk of kidney injury at 20 weeks post-RT. Sphingomyelin Phosphodiesterase Acid Like 3B (SMPDL3b) and Arginyltransferase 1 (ATE1) expression decreased more at 10 weeks after TBI followed by bone marrow transplantation in combination with CD4+ plus CD8+ T-cell treatment (BMT+T), as compared to focal bilateral kidney RT. TBI+T caused more normal tissue damage and inflammatory cytokine production compared to focal RT. As proposed in Aim 1.1, Rituximab pretreatment of wild type podocytes mitigated radiation-induced podocytopathy and increased cell survival. Related to Aim 2, double-strand DNA break (DSBs), measured by γH2AX, in wild type podocytes was maximal at 30 minutes post-RT and most breaks resolved within six hours. Fewer breaks were detected in SMPDL3b overexpressing podocytes two hours after exposure compared to wild-type podocytes. These data suggest that SMPDL3b is modulating the repair of radiation induced DSBs. An increase in Rad51 (Non-Homologous End-Joining; NHEJ) expression after 4 Gray (Gy) at up to 24 hours, while the expression of γH2AX and DNA-PKcs (Homologous Recombination Repair; HRR) decreased at 24 hours post-RT, indicating that the NHEJ mechanism of repair is of earlier onset and is more dominant than HRR in podocytes. We also investigated the radiation sensitivity of wild type, SMPDL3b overexpressing, SMPDL3b knock-out (KO) human podocytes, and Glomerular Endothelial Cells (GEC). GEC were the most sensitive to Room Temperature (RT) followed by KO podocytes. These data indicate that SMPDL3b deletion makes podocytes more sensitive to RT and that SMPDL3b overexpression protects podocytes against radiation injury. Since ultimately about 50% of cancer patients have radiation and many more also receive chemotherapy (bone marrow transplant and otherwise) that damage the kidneys by related mechanisms, understanding the mechanisms and developing approaches that reduce renal damage risk would substantively impact Floridians with cancer by improving patient quality of life, extending survival, and reducing health care costs.

   Follow-on Funding: None at the time of reporting

   Collaborations: None at the time of reporting

   Journals: None at the time of reporting

   Patents: None at the time of reporting

9. Grant #: 9LA09 Enhancing Immunotherapy Through Inhibition of Carbonic Anhydrase IX in Osteosarcoma

Principal Investigator: Julio Barredo, MD; Sulagna Banerjee, PhD

Organization: University of Miami

Grant Progress Report: Osteosarcoma is the most common bone tumor, primarily affecting children, adolescents and young adults, with the cure rate for this cancer being stagnated at about 70% for patients who have localized disease and below 30% in the fifth of patients who present with metastatic disease. Unfortunately, promising new treatments like immunotherapy, that has improved survival for several other cancers, have not worked well in Osteosarcoma. Hypoxic niches within the tumor contribute to therapeutic resistance in this disease. Hypoxia drives osteosarcoma cells to make more lactic acid, which eventually is effluxed from the tumor cells and rendering their surrounding acidic via Carbonic Anhydrase IX (CAIX). This further contributes to suppressing the immune system. Thus, inhibition of this acidification by targeting the CAIX proteins can promote the entry of cytotoxic immune cells and make osteosarcoma sensitive to immunotherapy. In collaboration with WeliChem BioTech, the research team has been testing the ability of their CAIX inhibitor, WBI5111 to sensitize against checkpoint inhibitors like anti-PD1 therapy. Over last year we have shown in one immune competent syngeneic model that mouse osteosarcoma cells K7M2 when injected in the tail vein, metastasize to the lungs. Treatment with CAIX inhibitor (100mg/kg, 5 days a week) has been started 10 days after implantation. We started the anti-PD1 injections at day 10 of the CAIX treatment, and administered three 100ug injections at days 10, 13 and 17. The animals were sacrificed after three weeks of treatment. Examination of the gross morphology of the lungs after fixing the tissue did not show in apparent difference in the control and treated groups. We next send the fixed samples for histological processing and stained them with HandE to evaluate the pathology and determine if there was a difference in the micro/macrometastasis. Results showed no quantifiable difference between the groups in micrometastatic lesions, while macro-metastatic lesions (lesions larger than 10 mm) seemed to decrease in combination group. Fetal human liver and thymus tissues were procured from the Advanced Bioscience Resources. Fresh liver tissue was divided into two parts and one part was used to purify CD34+ Hematopoietic Stem Cells (HSCs) using magnetic bead-based cell separation (Militenyi Biotech). The thymus tissue and the rest of the liver will be chopped into 1-3mm pieces and one piece of each was surgically implanted within the left subrenal capsule of each animal. Animals were followed for 12 weeks and the rate of humanization was checked in three animals by flow cytometry using human CD45+ antibody (40-45% humanized). Human osteosarcoma cells SaOS2 were injected in the lung through tail vein. After five days the animals were randomized and put into four treatment groups: Control, WBI-5111, anti-PD1 ab, anti-PD1+ WBI-5111. The animals were closely monitored. The experimental design. Analysis of the blood showed good humanization. Normally more than 25% humanization is considered to be a good engraftment. Our model had up to 80% engraftment by the end of the experiment. We next studied the survival of the animals receiving the treatment. Our results showed that that the mice receiving combination therapy had a better survival rate than animals receiving the individual drugs. Representative pictures of tumor bearing lungs from the control group and WBI-5111 group can also be seen.

Since the departure of Dr. Trucco, the team has had difficulty getting patient tissues. An MTA for procurement of osteosarcoma samples from the NIH PDX core (that has several samples) is in process, however, owing to the delays posed by the COVID#19 pandemic, it may be delayed. The team also looking for alternate sources. Challenges were faced in setting up the breeding

for the transgenic mice. The team has decided to evaluate our finding in syngeneic mouse models instead using osteosarcoma orthotopic models. All these cells are very slow growing and take up to 4 weeks to form measurable tumors. This significantly slows down the experimentation. Intra-osseous models. The lab has been closed since March 15, 2020 because of the pandemic. This is significantly affecting the progress of research in this project.

10. Grant #: 9LA10 Anti-Neuroblastoma Effects of ICA-1

Principal Investigator: Mildred Acevedo-Duncan, PhD

Organization: University of South Florida

Grant Progress Report: Neuroblastoma, the pediatric cancer of the early nerve cells, has the tendency to become resistant to traditional therapeutics due to its highly heterogeneous nature. Atypical Protein Kinase C-iota (PKC-ι) has been found to promote cell proliferation in neuroblastoma cells through the PKC-ι /Cdk7/Cdk2 signaling pathway. The research team sought out to ascertain the potential of aPKC inhibitors, 5-amino-1-2,3-dihydroxy-4-(methylcyclopentyl)-1H- imidazole-4-carboxamide (ICA-1S) (PKC-ι specific) and 8-hydroxy-1,3,6-naphthalenetrisulfonic acid (ζ-Stat) (PKC- ζ specific), as neuroblastoma therapeutic agents. Various experimental studies were carried out based on key metrics of drug functionality, including; toxicity, cell proliferation and effects on molecular pathways. To that end, this study utilized the neuroblastoma cell lines; I-type (intermediate) CHP-212and BE 2)-C, N-types (neuroblastic); BE (2)-M17and SH-SY5Y and S-types (substrate adherent); SK-N-BE(2) and LAI-5s, representing neuroblastoma heterogeneity. The inhibitors, ICA-1S and ζ-Stat showed cell to cell line variation in their toxicity, effect on proliferation and molecular marker expression. ICA-1S displayed the most toxic effects on BE(2)-C as well as showing the ability to decrease the proliferation of both I-type cell lines with increased concentration. The N-type cell lines showed differing responses to inhibitors with BE(2)-M17 showing a greater susceptibility to inhibitors than SH-SY5Y. Finally, the S-type and least malignant of the types displayed low changes when treated with the inhibitors. Western blot analysis was performed to assess how the inhibitors effected various molecular targets and by extension various signaling pathways. These results gave a lot of insight into the signaling pathways shared by and those varying amongst the various cell types. In most cases both the I and N-type cell lines displayed increases in apoptotic markers and decreases in mesenchymal markers with the S type cell lines showing slight changes in marker expression. In previous reports the team displayed the downregulation of vimentin among those mesenchymal markers. Further exploration of vimentin downregulation as an effect of the inhibitors usage by observing their effects at established serine phosphorylation sites; 33,39 and 56. The team gained further information as to the cell cycle and the link to PKC-ι phosphorylation. The research team demonstrated that PKC-ι may be phosphorylated at key points in the cell cycle based on cell line, and also established that changes the PKC-ι /Cdk7/Cdk2 pathway in BE (2)-C occurred across two other cell lines, BE (2)-M17 AND CHP-212, though in varying ways further backing the heterogenous nature of neuroblastoma. The use of immune-fluorescent microscopy and quantification was used to gain visual insight of the inhibitor effects on molecular markers. During the quarter our poster “Atypical PKC inhibitors ICA-1S and ζ-Stat show inhibition of neuroblastoma cell proliferation” was presented at the virtual meeting off the AACR with the citation placed in the appropriate position in this report. Difficulties came mainly in the form of the optimization of new techniques and antibodies and the delay in reagent delivery services due to the current pandemic. In the immunofluorescent experiment the team will be delving into the literature to further optimize

getting the antibody into the cells.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting

Live Like Bella Pediatric Cancer Research Initiative
Appendix U
Fiscal Year 2019-2020 Active Grants
Funding Fiscal Year 2017-2018

Grant #	Organization	Principal Investigator	Award Amount	Life to Date Expenditure	Unspent Funds	Executed Date	End Date	Patents	Publications	Follow-on Funding
8LA01	University of Florida	Jonathan D Licht, MD	$200,000	$127,773.00	$72,227.00	5/11/2018	4/30/2021	No	No	Yes
8LA02	University of Central Florida	Cristina Fernandez-Valle, PhD	$200,000	$144,439.00	$55,561.00	6/14/2018	4/30/2021	No	No	No
8LA04	Baptist Health South Florida	Matthew Hall, MBA, MD	$700,000	$379,166.58	$320,833.42	6/14/2018	4/30/2022	No	No	Yes
8LA05	Florida International University	Diana Azzam, PhD	$700,000	$379,166.00	$320,834.00	5/11/2018	4/30/2022	No	No	No

1. **Grant #:** 8LA01 Identification of Therapeutic Targets and Pathways in Relapsed Childhood Acute Lymphocytic Leukemia Associated with NSD2 Mutation

   Principal Investigator: Jonathan D. Licht, MD

   Organization: University of Florida

   Grant Progress Report: Progress has been made on all aspects of the proposal. In the first aim the research team examined the spectrum of drug resistance in acute lymphocytic leukemia cells which harbor the NSD2 activating mutation found particularly in children with relapsed ALL. Our data showed that the NSD2 mutant ALL cells were sensitive to most chemotherapy agents but were resistant to glucocorticoids (GC). Further study showed that the NSD2 mutation led to depressed levels of the glucocorticoid receptors (GR) in leukemia cells, and a failure of GC to amplify the expression of the GR and allow GR binding to critical genes responsible for killing the leukemia cell. Furthermore, our team found that pretreatment of the NSD2 mutant ALL cells with an inhibitor of the EZH2 repressive chromatin modifying enzyme then once again allowed the GR to be induced by GC. GR levels rose and could bind to targets and kill leukemia cells. When this combination treatment was applied to mice harboring the NSD2 mutant leukemia cells lifespan was extended in the mice by 4-6 weeks. This could be a potential treatment for patients with this form of ALL since EZH2 inhibitors are approved for other clinical indications.

   The second part of the proposal aims to find the key gene(s) that cause the aggressive brain invading behavior of NSD2 mutant leukemia. Screening began on cells for genes that are critical for invasion and motility of tumor cells and identified several candidate genes which will now be validated. A more extensive library is being created to disrupt over 1000 genes regulated by mutant NSD2 to see which of these genes are most critical for cell growth and invasion. Collectively our findings suggest a new therapeutic approach for relapsed ALL associated with NSD2 mutation: pre-treatment with an EZH2 inhibitor followed by glucocorticoids. Secondly, the team is identifying genes downstream of NSD2 responsible for the aggressive biological behavior of these tumors which might represent additional therapeutic targets. There have been no institutional changes to affect the research. A poster on this work was presented at the 2019 Society of Hematology (ASH) annual meeting and the PI presented the research at a seminar at Loyola University Medical Center, Chicago in 2020. The team is in the final stages of finishing a manuscript on NSD2 mutation and GC resistance.

   Follow-on Funding: Rally Foundation; The Role of NSD2 Mutation in Therapy Resistance in Childhood ALL; Jianpin Li, MD; $50,000
   PROPOSAL/GRANT TITLE: *The role of NSD2 mutation in therapy resistance in childhood ALL*

   Collaborations: Collaboration with Cold Spring Harbor Labs (NY) in the construction of a library of guide RNAs directed against NSD2 targets.

   Collaboration with the NIH Center for Advancement of Translational Science (NCATS) on drug screening in *NSD2* mutant ALL cell lines.

   Collaboration with Dr. Richard Lock (Children's Cancer Institute, Sydney, Australia) on ALL patient-derived xenograft (PDX) experiments.

   Journals: Manuscript ***"A Gain of Function Mutation in the NSD2 Histone Methyltransferase Drives Glucocorticoid Resistance of Pediatric Acute Lymphoblastic Leukemia"*** **is under**

preparation for submission recently.

ASH Abstract cited by:
Li B, Brady SW, Ma X, et al. Therapy-induced mutations drive the genomic landscape of relapsed acute lymphoblastic leukemia. Blood. 2020 Jan 2;135(1):41-55. doi: 10.1182/blood.2019002220.

Wandler AM, Huang BJ, Craig JW, et al. Loss of glucocorticoid receptor expression mediates in vivo dexamethasone resistance in T-cell acute lymphoblastic leukemia. Leukemia. 2020 Feb 17. doi: 10.1038/s41375- 020-0748-6. [Epub ahead of print]

Patents: The "EZH2 and EZH2/PRC2 inhibitors to reverse glucocorticoid resistance in NSD2 mutant acute lymphocytic leukemia," invention disclosed to the University of Florida

2. Grant #: 8LA02 Synergistic PI3K Combinatorial Targeting for NF2 Schwannoma

Principal Investigator: Cristina M. Fernandez-Valle, PhD

Organization: University of Central Florida

Grant Progress Report: The purpose of this research is to identify two compound and/or drugs that work together to reduce the growth and survival of schwannoma cells. Schwannomas are benign tumors that can form in any nerve in the body in children with Neurofibromatosis Type 2 (NF2). This is a rare disorder affecting one of every 40,000 individuals. There is currently no drug treatment for NF2. Surgical removal of schwannomas causes loss of movement and/or sensation of the body part controlled by the affected nerve. Although schwannomas can grow in any nerve, all NF2 patients have schwannomas on the hearing and balance nerve within the head. NF2 patients typically become deaf as young adults. Radiation is not recommended as the risk of a future malignant tumor increases. Effective compounds/drugs must target cell communication pathways used by schwannoma cells to grow and/or live. Previous work identified phosphoinositide 3-kinase (PI3K) as a major pathway to target with drugs. However, schwannoma cells, as do many cancer cells, increase activity of other pathways to compensate for loss of PI3K activity when exposed to PI3K inhibitors. Therefore, an effective drug treatment will likely require using two drugs. The goal of the second year of this award was to test two drugs alone and together in a mouse model of NF2 schwannomas. The drugs selected based on year one results, were pictilisib, a PI3K inhibitor and PF-758309, a p-21 activated kinase (PAK) inhibitor. The progress this year has been to complete the drug efficacy study. This entailed completing two pharmacokinetic studies to establish whether drugs entered the nerves, confirm dosing regimens, and finally to measure drug levels in tumors from treated mice. Tumor growth rate in 48 mice was measured in two ways: final tumor weight and bioluminescence at three time points. The most significant finding was that pictilisib is permeable to the blood nerve barrier and alone reduced graft size by 66% of vehicle control levels ($p=0.01$). Our drug dosing level was equivalent to the reported effective dose in patients in a phase 1 clinical trial for cancer. Pictilisib is by far the most effective PI3K inhibitor tested by us in this NF2 schwannoma mouse model. Three other PI3K inhibitors have been tested. This observation warrants further study. There was no benefit of the drug combination in further reducing tumor size beyond that observed with pictilisib alone. Although, PF-758309 also reduced graft size by 44% of control levels, the reduction was not statistically significant. Additional studies could be warranted pending results of the immunohistochemical assessment of grafts. The long-term impact of the work on the health of children with NF2 is to identify: 1) critical pathways used by schwannoma cells to survive and grow that should be targeted by a drug therapy, and 2): compounds or

current cancer drugs that can be repurposed for treatment of schwannomas. The research also contributes in training future researchers and medical professionals in an understanding of this rare tumor disorder and in technical procedures used in biomedical research.

Follow-on Funding: None at the time of reporting

Collaborations: All the training has been conducted in the College of Medicine, Burnett School of Biomedical Sciences, Divisions of Neuroscience, and Cancer Research working with post-doctoral fellows and undergraduate students at the Health Science Campus of the University of Central Florida in Orlando, FL.

Dr. Berta Victoria was a post-doctoral fellow for two years. Dr. Victoria was supported by this award and a post-doctoral award from the University of Central Florida. Dr. Victoria conducted the animal drug efficacy study aided by Mrs. Rosa Rosario. Dr. Victoria finished her training in February 2020 .

Rosa Rosario is a senior technician specialized in animal husbandry and is partially supported by this award. Ms. Rosario conducted the in vivo efficacy study and continues working on tissue analysis.

Dr. Alejandra Petrilli, a senior scientist in the lab. Dr. Petrilli stepped in to keep the project moving forward during the COVID shutdown. Dr. Petrilli assisted in analysis of pharmacokinetic data and optimizing the histological stains and immunohistochemistry protocols. Dr. Petrilli is training personnel on immunohistochemistry and is imaging slides.

Biostatistics Core: The pharmacokinetic results and in vivo drug efficacy results were analyzed by Mr. Matthew Robinson and Mr. Shawn Zhu. Both have Masters' degrees in statistics.

Four undergraduate students of the University of Central Florida in the Burnett School of Biomedical Sciences received or continue to receive research training related to this project. Andrew Tritran an undergraduate student who provided support during the in vivo drug study.

Abdul Allaf has trained in my lab for nearly three years and conducted the quality control of the mouse schwannoma model cells implanted into immunocompromised mice.

Eliel Ruiz was an undergraduate student receiving federal support through the work-study program. He was already experienced in research and assisted in the in vivo drug study by organizing protocols and supporting sample collection at the end of the study and fixing and embedding the tissue.

Michael Dang is an undergraduate student who provided support in tissue collection, embedding, sectioning and is training in immunohistochemistry.

Journals: A poster was presented at the virtual annual international conference on Neurofibromatosis organized by the Children's Tumor Foundation. Synergistic Combinatorial Phosphatidylinositol-3-Kinase (PI3K) Targeting for NF2 Schwannoma. Alejandra M. Petrilli, PhD, Marga Bott MS, Berta Victoria PhD, Rosa Rosario BS, and Cristina Fernández-Valle PhD, University of Central Florida.

Patents: None at the time of reporting

3. **Grant #:** 8LA04 The Impact of Radiation Dose on Brain Morphology, Volumetric Changes, Endocrine Function, and Neurocognitive Function Following Cranial Radiation Therapy in Children with Brain and Skull Base Tumors

   Principal Investigator: Matthew D. Hall, MBA, MD

   Organization: Miami Cancer Institute; Baptist Health South Florida Department of Health

   Grant Progress Report: Most brain tumor patients are treated with surgery, chemotherapy, and radiotherapy, which can all cause significant harm to memory, hormone function, and functional independence. In a large study of pediatric patients treated before 1996, more than 1/3 of childhood brain tumor survivors were unable to finish school, live independently, and maintain employment. New strategies are needed to reduce the negative effects of treatment and enable more brain tumor survivors to lead independent and productive lives following curative treatment.

   The aims of this trial are to evaluate the changes in the brain following cranial radiation therapy in pediatric cancer patients with brain or skull base tumors and to identify and measure cause and effect relationships between the treatment delivered to critical brain substructures and the resulting effects on cognition, endocrine function, quality of life, and productivity.

   Since study activation in 2018, 34 patients (of the planned 60) have enrolled on this study and about 20 completed at least 6 months of follow-up after radiotherapy. During early follow-up, the research team has identified that parts of the brain receiving high dose radiation can demonstrate measurable shrinkage as early as 6 months following treatment. At 6 months, no clear negative effect has been observed on cognitive testing scores, which likely take longer to develop. Of particular interest, patients who have been most likely to report difficulties in school and adverse quality of life are those who developed toxicities after treatment, which deserves attention during continued follow-up. With this grant funding, the research team will follow patients for at least two years after treatment to track the effects of radiotherapy. Long-term results are eagerly anticipated as this trial matures. Future reports will detail the observed effects of radiation and other variables on memory, school performance, hormone function, and quality of life with longer time elapsed after treatment completion. Also, the research team has secured additional funding to follow patients for an additional three years (with patient consent), further increasing potential learning and the scientific impact of this study, which were enabled by support from this grant.

   The research team has shown early proof of principle that changes in the size of critical brain substructures are observed after radiotherapy and can be measured using a novel software package, which is the primary aim of this trial. With longer follow-up (and time to observe the adverse effects of treatment), the data collected from these patients will help better quantify health outcomes following radiotherapy and guide protection strategies to minimize harm from radiation. The research team finds that more frequent cognitive testing and quality of life assessments in patients treated on this study can identify potential problems and troubleshoot them sooner. The close follow-up model developed for this project is now used on all adult/pediatric brain tumor patients treated with curative intent at our institution (estimated at 200 patients per year) to promote better quality of life and survival.

   Follow-on Funding: Baptist Hospital Foundation; Follow-up on patients who agree to continue with additional research measurements following the 2-year time horizon supported by Live Like Bella Grant Funding; $100,000

Collaborations: Warren Rehrer, BS, who is a medical student at Florida International University College of Medicine, Miami, FL and a research collaborator at Miami Cancer Institute, Miami, FL has received training and participated in research on this project. The principal investigator gratefully acknowledges the hard work performed on this project in terms of data collection/database entry and thoughtful insights on research inquiries and hypotheses.

The research team also gratefully acknowledges the contributions of our research collaborators, including:

- **a.** Dr. Reshma Naidoo in the Department of Pediatric Neuropsychology at Nicklaus Children's Hospital, Miami, FL, and Dr. Richard Hamilton and Ms. Aileen Moreno in the Department of Supportive Care Medicine at Miami Cancer Institute, who have worked together to provide neurocognitive testing for all patients on this study.
- **b.** The Principal Investigator would specifically like to acknowledge Ms. Golnar Alamdari, a Student Intern in Pediatric Neuropsychology at Nicklaus Children's Hospital who has participated in the testing of many patients as part of training.
- **c.** Dr. Toba Niazi in the Department of Pediatric Neurosurgery at Nicklaus Children's Hospital who has been instrumental in-patient referral, clinical care, and evaluation of study MRIs.
- **d.** Dr. Ossama Maher in the Department of Pediatric Hematology/Oncology at Nicklaus Children's Hospital and Mrs. Katherine Von Werne in the Department of Radiation Oncology at Miami Cancer Institute, who have been especially helpful in ensuring patients receive the necessary follow-up studies for this trial.
- **e.** Dr. Kevin Abrams in the Department of Radiology at Miami Cancer Institute who has been instrumental in setting up the MRI scan parameters at Miami Cancer Institute, Nicklaus Children's Hospital, and all participating imaging centers where patients have had MRIs performed on this trial to ensure that the necessary data is collected for research purposes.

Journals: None at the time of reporting

Patents: None at the time of reporting

4. **Grant #:** 8LA05 Personalized Ex Vivo Drug Screening and Genomics Profiling to Guide Individualized Treatments for Children with Relapsed or Refractory Solid Tumors and Leukemias

   Principal Investigator: Diana Azzam, PhD

   Organization: Florida International University

   Grant Progress Report: This study is a multi-center clinical trial between Florida International University and Nicklaus Children's Hospital. The purpose of this feasibility study is to provide novel therapeutic options for children with relapsed and/or refractory cancers using our robust high throughput ex vivo drug sensitivity testing (DST) and combining it with mutation analysis. The functional drug screening test (DST) panel encompassed 40 formulary drugs frequently used at Nicklaus Children's hospital and 47 non-formulary drugs approved by FDA for cancer treatment as well as drugs from phase III and IV clinical trials. Drug sensitivity score (DSS) was calculated for each drug based on cancer cells' response. DST results were then combined with results from the genetic screen to match actionable mutations with selective targeted therapies. The main outcome of the study is the proportion of the patients whose treatment was guided by

the personalized medicine approach.

Since December 2018 till June 2020, the research team has successfully consented and recruited a total of ten patients with recurrent/refractory cancers. Out of the ten patients, we were able to perform DST and/or mutation profiling on nine patients. Most importantly, we optimized and successfully performed our drug sensitivity assay on at least six different tumor types including leukemia, osteosarcoma, rhabdomyosarcoma, Ewing's sarcoma, glioblastoma and lung. Fresh tumor samples from nine patients with ex vivo DST returned between 10-30 treatments options for each patient. These patients showed vastly different responses to the 103 FDA-approved compounds used in the screen. Indeed, no compound showed activity in all of the patients, but more than half of the evaluated compounds were not active in any of the patients. With these results, six patients were treated on DST-guided protocols and four have showed complete objective response, so far. Progression-free survival and overall response to DST-guided treatment is being as compared with their own progression-free survival for the most recent regimen on which they had progressive disease. Overall, the feasibility of this methodology in children to identify candidate agents with clinical potential has been confirmed. DST provided valuable information to the oncologists on drug dosing and treatments that may not be effective and should be avoided. The team would like to continue to implement this novel and personalized approach to assess clinical response and progression-free survival.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: None at the time of reporting

Patents: None at the time of reporting