Biomedical Research Advisory Council

Bankhead-Coley Cancer Research Program

James and Esther King Biomedical Research Program

Live Like Bella Pediatric Cancer Research Initiative

2018-2019 Annual Report

Ron DeSantis
Governor

Scott A. Rivkees, MD
State Surgeon General

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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) 2018-2019, 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.

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 2018-2019, 10 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 statute requirements, a 2018-2019 fiscal-year 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; (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.

- 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, second to heart disease. 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 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.

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.

BIOMEDICAL RESEARCH GRANT ADVISORY BOARD OVERVIEW AND MEMBERSHIP

The Biomedical Research Advisory Council (section 215.5602, 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:

- Providing advice on program priorities and emphases.
- Providing advice on the overall program budget.
- Participating in periodic program evaluation.
- 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.
- Assisting in the development of appropriate linkages to nonacademic entities, such as voluntary organizations, health care delivery institutions, industry, government agencies, and public officials.
- Developing criteria and standards for the award of research grants.
- Developing guidelines relating to solicitation, review, and award of research grants and fellowships to ensure an impartial, high-quality peer review system.
- Reviewing reports of peer review panels and making recommendations for research grants and fellowships.

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, Ph.D. (Chair), Professor and Associate Chair, Pediatrics; Director, Mailman Center for Child Development, University of Miami Miller School of Medicine; Seat: American Cancer Society

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

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

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

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

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

Tushar Patel, M.B., Ch.B., Dean of Research, Mayo Clinic; Seat: Senate

Michael Fradley, M.D., Assistant Professor, University of South Florida College of Medicine, USF South Tampa Center; Seat: House of Representatives

Conor Lynch, Ph.D., Associate Member, Moffitt Cancer Center; Seat: House of Representatives

Mary P. Martinasek, Ph.D., M.P.H, Associate Professor and Assistant Dean of College of Natural and Health Science, University of Tampa; Seat: American Lung Association

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 & 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%, breast cancer by 15%, prostate cancer by 20%, colon cancer by 25%, and melanoma by 15% 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, 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% increase in early detection of cancer or preventable cancer within 10 years.

- Technology Transfer Feasibility

  - 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%, breast cancer by 30%, prostate cancer by 30%, colon cancer by 30%, and melanoma by 30% 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% and adults to less than 15% 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.

Fiscal Year 2018-2019 funding cycle awards were made to support the following research priorities for Bankhead-Coley, King and Bella grants:

25 Awards – Prevention and Treatment: (11 Bankhead-Coley, 7 King, 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.

1 Award – Technology Transfer Feasibility (TTF): (1 Bankhead-Coley) 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.

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

3 Awards – Health Disparities: (1 Bankhead-Coley,1 King, 1 Bella) 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 – Screening: 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: (1 Bankhead-Coley, 2 King, 2 Bella) 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, central nervous system, reproductive, developmental impairment, Graft-versus-host disease).

1 Award – Investigational New Drug (IND) or Investigational Device Exemption (IDE): (1 Bankhead-Coley) The goal of this mechanism is to expand upon research that supports the development of Investigational New Drug and Investigational Device Exemption 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.

Figure 1: Bankhead-Coley Applications and Funded Projects

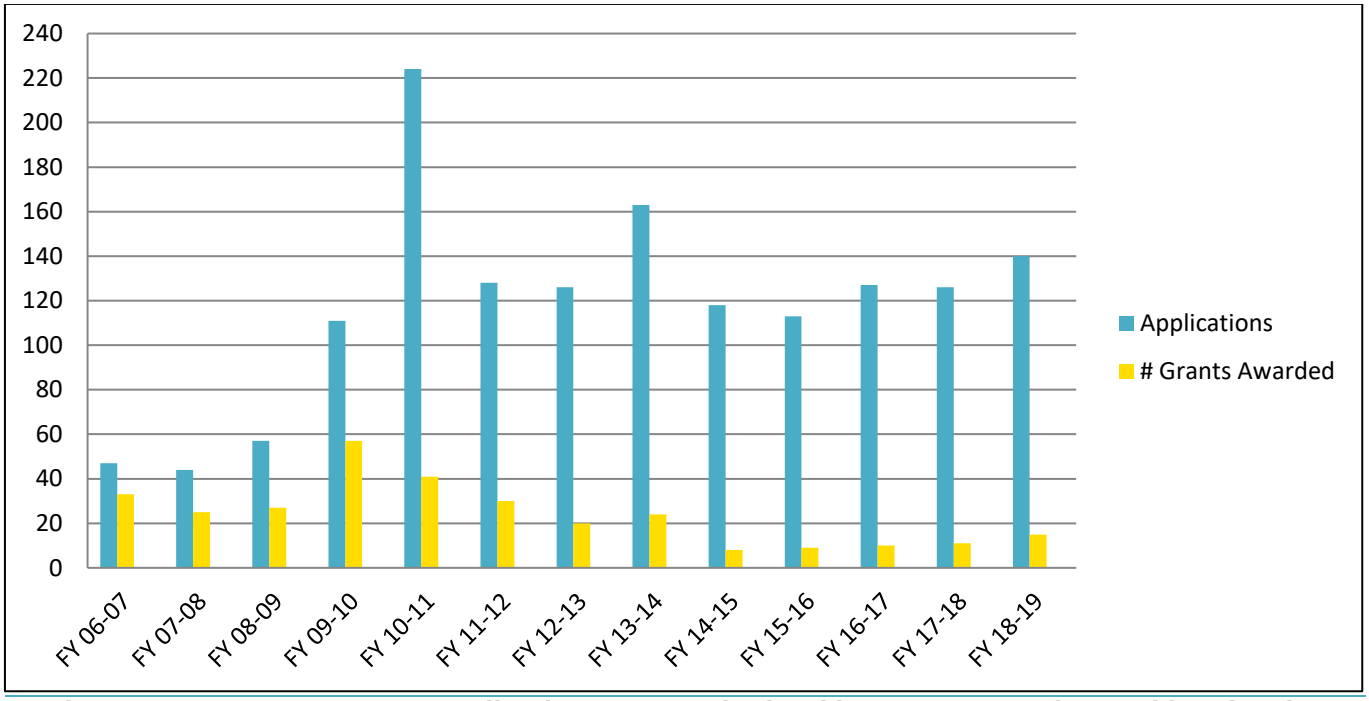

Figure 1: In 2018, 140 grant applications were submitted in response to the Bankhead-Coley funding opportunity announcement. The FY 18-19 was allocated to 15 cancer-related disease research projects.

Figure 2: King Applications and Funded Projects

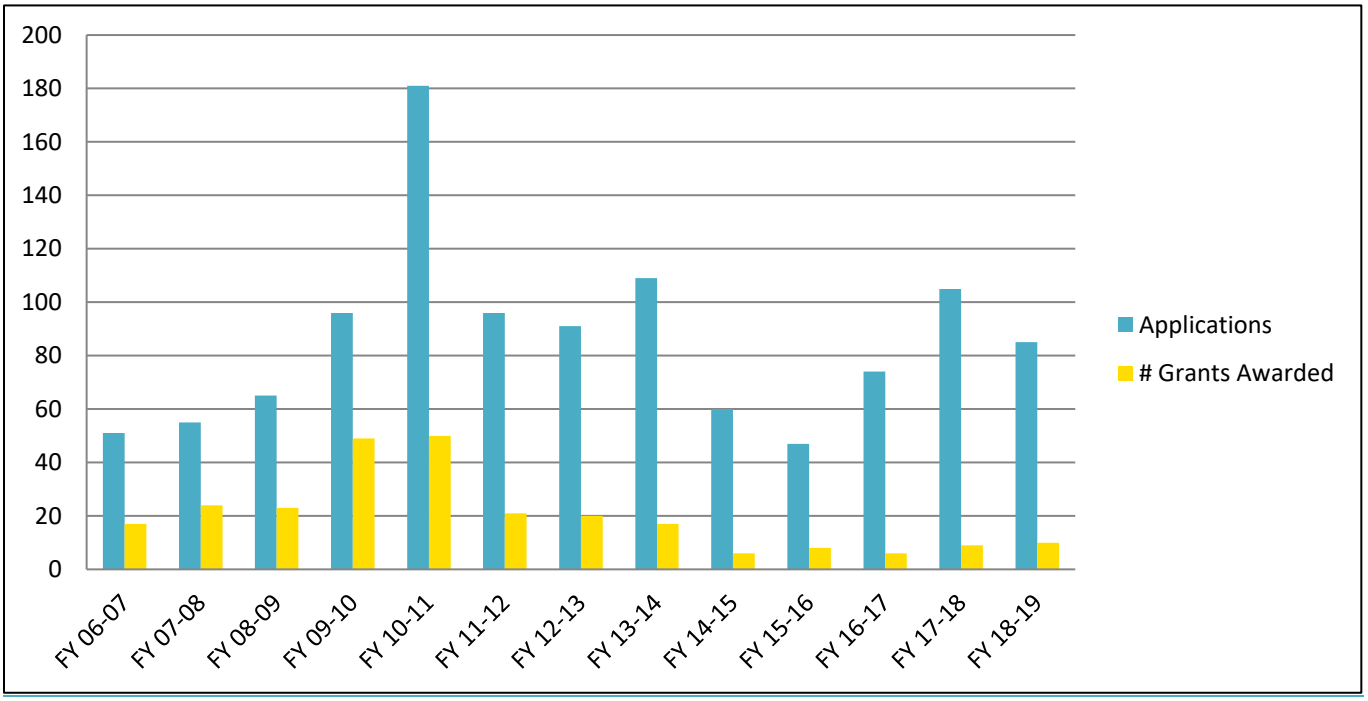

Figure 2: In 2018, 85 grant applications were submitted in response to the King funding opportunity announcements. The FY 18-19 funding was allocated to 10 tobacco-related disease research projects.

Figure 3: Bella Applications and Funded Projects

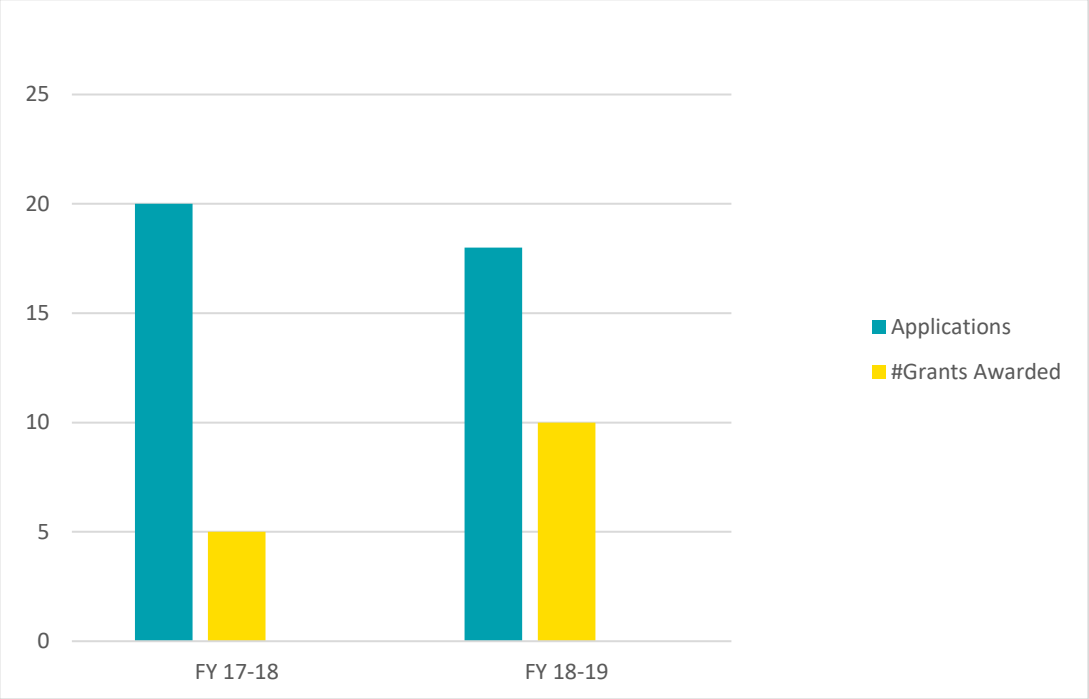

Figure 3: In 2018, 18 grant applications were submitted in response to the Bella funding opportunity announcement. The FY 18-19 was allocated to 10 pediatric cancer research projects. As the program continues to become known, it is anticipated that more grant applications will be submitted.

NATIONAL INSTITUTES OF HEALTH (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. While remaining in 12th, there was a decrease in the total amount of funding for FY 2018-2019.

National Institute of Health Biomedical Research State Funding and Rankings Fiscal Year 2018

State	Total Funding	Rank
CA	$4,243,446,496	1
MA	$2,887,150,148	2
NY	$2,632,652,693	3
PA	$1,810,217,516	4
MD	$1,531,640,271	5
NC	$1,402,438,380	6
TX	$1,243,375,373	7

WA	$1,042,298,686	8
IL	$895,375,844	9
OH	$816,911,217	10
MI	$766,267,407	11
FL	$607,699,382	12
MO	$605,505,650	13
GA	$582,663,762	14
MN	$561,671,411	15
CT	$560,899,834	16
TN	$549,653,495	17
WI	$481,664,069	18
VA	$415,082,162	19
CO	$404,428,934	20

Figure 4: NIH Research Funding from the 2018 Fiscal Year Reporting Period. The top twenty states in NIH funding is displayed. With over $607 million in NIH funding, Florida is ranked 12th in the nation. *Source: NIH Research Portfolio Online Reporting Tools (RePORT)*

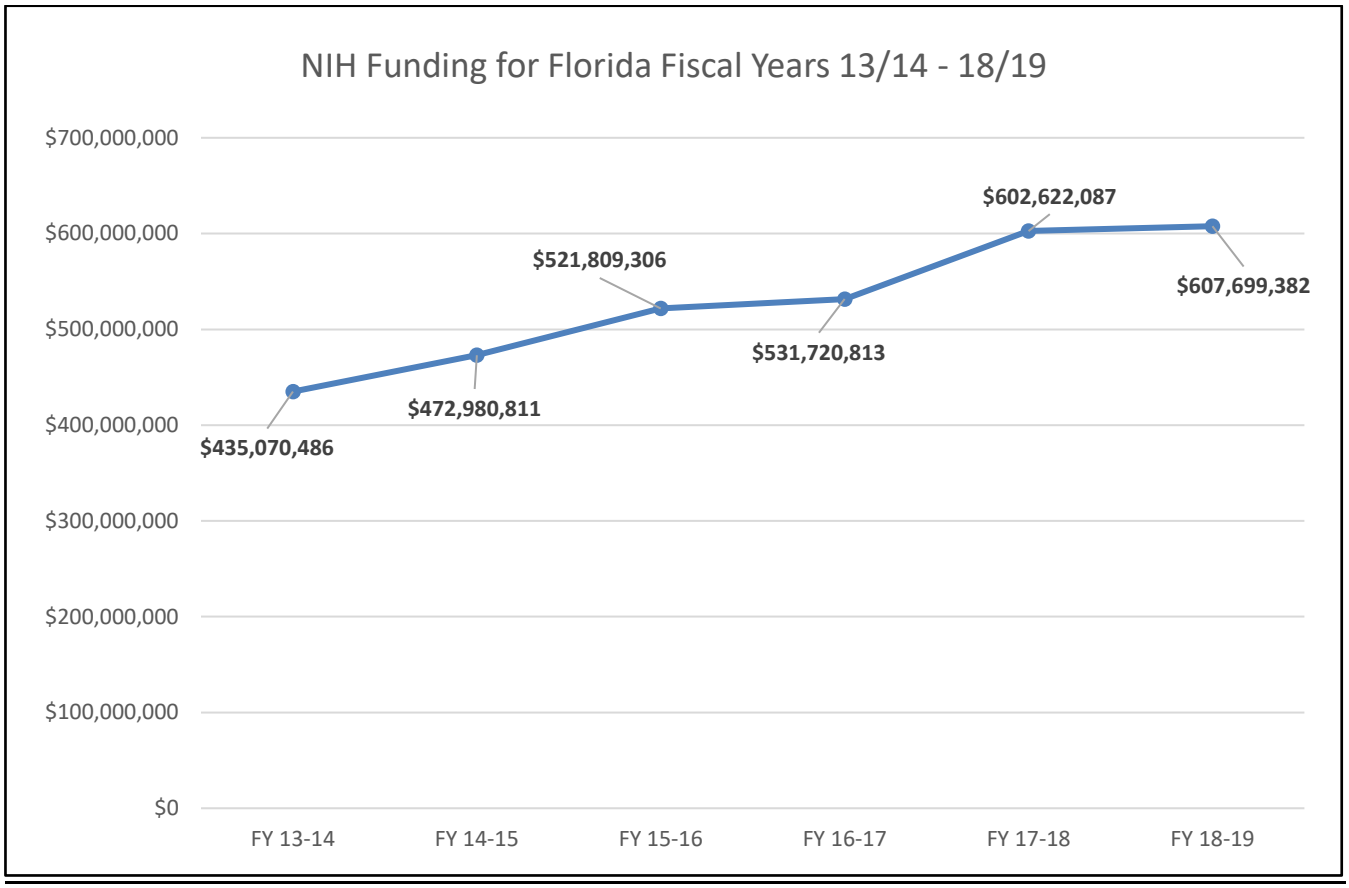

Figure 5: NIH Funding for Florida has remained over $600M in the last two years. These results reflect Florida's initiative to expand upon research to improve scientific understanding of various diseases and health disparities.

Bankhead-Coley Cancer Biomedical Research Program

APPENDIX A

FISCAL YEAR 2018-2019 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
9BC01	Florida Atlantic University	Guzman, Esther	$ 801,000	$0.00	$ 801,000.00	5/10/2019	5/31/2022	No	No	No
9BC02	Florida Atlantic University	Lu, Michael	$ 58,162	$0.00	$ 58,162.00	5/30/2019	12/15/2019	No	No	No
9BC03	Florida State University	Steiner, Jennifer	$ 732,238	$0.00	$ 732,238.00	6/17/2019	5/31/2022	No	No	No
9BC04	Florida State University	Rust, George	$ 800,487	$0.00	$ 800,487.00	5/28/2019	5/31/2022	No	No	No
9BC05	H. Lee Moffitt Cancer Center	Rejniak, Katarzyna	$ 100,000	$0.00	$ 100,000.00	3/22/2019	9/30/2019	No	No	Yes
9BC06	H. Lee Moffitt Cancer Center	Rodriguez, Paulo	$ 100,000	$0.00	$ 100,000.00	3/22/2019	9/30/2019	No	No	No
9BC07	H. Lee Moffitt Cancer Center	DeNicola, Gina	$ 1,335,000	$0.00	$ 1,335,000.00	4/17/2019	5/31/2024	No	No	No
9BC08	H. Lee Moffitt Cancer Center	Bejanyan, Nelli	$ 1,335,000	$0.00	$ 1,335,000.00	4/17/2019	3/31/2024	No	No	No
9BC09	H. Lee Moffitt Cancer Center	Schonbrunn, Ernst	$ 800,454	$0.00	$ 800,454.00	3/26/2019	3/31/2022	No	No	No
9BC10	Mayo Clinic	Storz, Peter	$ 85,409	$0.00	$ 85,409.00	3/26/2019	9/30/2019	No	No	Yes
9BC11	University of Miami	Lossos, Izidore	$ 100,000	$0.00	$ 100,000.00	6/04/2019	10/31/2019	No	No	No
9BC12	University of Miami	Capobianco, Anthony	$ 801,000	$0.00	$ 801,000.00	5/07/2019	4/30/2022	No	No	No
9BC13	University of Miami	Burnstein, Kerry	$ 801,000	$0.00	$ 801,000.00	4/23/2019	4/30/2022	No	No	No
9BC14	University of South Florida	Jiang, Rays	$ 801,000	$0.00	$ 801,000.00	6/04/2019	4/30/2022	No	No	No
9BC15	University of South Florida	Kim, Minjung	$ 100,000	$0.00	$ 100,000.00	5/29/2019	10/31/2019	No	No	Yes

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

Principal Investigator: Esther A. Guzmán, PhD

Organization: Florida Atlantic University

Abstract of Proposed Research: Guzmán and her team are investigating the use of marine natural compounds to treat triple negative breast cancers. Triple negative breast cancers, which represent about 12% of breast cancers diagnosed in the United States, can be very aggressive and easily spread to other organs, particularly the brain and the lungs. They are more likely to recur than other breast cancers and are classified as high-grade tumors because of the minimal resemblance these cancer cells have to normal cells.

The objective of this research is to identify compounds from the extensive marine natural products library at Florida Atlantic University's Harbor Branch Oceanographic Institute that can induce programmed cell death (apoptosis) in triple negative breast cancer cells grown as spheroids. Cells grown as spheroids more closely mimic tumors, and, therefore, are expected to be more easily translated to the clinic. The hope is that these clinically active compounds will provide more effective treatment options for triple negative breast cancers, with less side effects and greater survival rates. The five-year survival rate for triple negative breast cancers is 77% compared to 93% for other breast cancer types.

Guzmán and her colleagues will use a multi-parametric cell-based assay that uses high-content imaging to measure the cell number, induction of apoptosis and viability in two triple negative breast cancer cell lines grown as spheroids. So far, the research team has purchased both cell lines, created frozen stocks and determined the fractions that will be tested. It also has obtained quotes for several of the reagents which are in the process of being purchased. Additionally, researchers have established an account with MD Anderson Cancer Center so they can send samples to be analyzed by the center's Reverse Phase Protein Array (RPPA) Core Facility.

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 #9BC02: PAK6 in Advance Prostate Cancer

Principal Investigator: Michael Lu, PhD

Organization: Florida Atlantic University

Abstract of Proposed Research: Despite clinical evidence indicating that androgen promotes the metastatic progression of prostate cancer cells, the underlying mechanism remains unclear. The current proposal aims to study a novel signal molecule p21-activated kinase 6 (PAK6) that functions as a hormone-regulated dominant factor that contributes to treatment-induced drug resistance in advanced prostate cancer. The identification of PAK6 activation as an androgen-stimulated androgen receptors

(AR)-mediated event suggests a potential target for intervention of hormone-regulated prostate cancer metastasis. The researchers have determined that PAK6 is pivotal to the development of treatment-induced drug resistance in neuroendocrine (NEPC) advanced prostate cancer. The data indicate PAK6 expression in advanced prostate cancer promotes drug resistance by downregulating Hippo pathway and upregulating *wingless/int-1* class (Wnt) signaling. The characterization of PAK6 as a therapeutic target to mitigate the treatment induced drug resistant in prostate cancer will pave the way to a novel modality in combating this dreadful disease.

Follow-on Funding: None at the time of reporting.

Collaborations: Ole Gjoerup, Collaborator, Dana Farber Cancer Institute, Harvard Medical School

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Jennifer Steiner, PhD

Organization: Florida State University

Abstract of Proposed Research: Colorectal cancer is among the most prevalent cancers and is the second leading cause of cancer-related death in the United States. Frequently drinking moderate (fewer than one-to-four drinks per day) and high (four or more drinks per day) levels of alcohol increases colon cancer risk. In Florida, drinking prevalence was above the national average as 24% of people aged 65 and older reported higher levels of alcohol intake, classified as 10 or more drinks a week for men or seven or more drinks a week for women. This is concerning as cancer prevalence in Florida is the second highest in the nation, and colon cancer risk increases with age. While the health implications of a cancer diagnosis are obvious, accompanying comorbidities, like cancer cachexia, lead to additional health concerns. Cancer cachexia occurs in about 50% of colon cancer patients and is characterized by the loss of skeletal muscle and fat mass directly contributing to decreased muscle strength, quality of life and treatment compliance and efficacy as well as increased mortality. Cachexia can develop at any point in the disease process but typically worsens either as the tumor burden increases or during chemotherapy. Lifestyle may also influence cachexia risk and development. For example, alcohol was recently shown to worsen cachexia and increase mortality in a mouse model of melanoma. Much remains to be learned about how alcohol contributes to cachexia especially in relation to the loss of skeletal muscle. Therefore, the main objectives of this work include: 1) Determination of the molecular factors enhancing the loss of muscle at each stage of cachexia when alcohol is consumed before and/or during colon cancer; 2) Test whether alcohol worsens cachexia during chemotherapy treatment with 5-flurouracil (5-FU); and 3) Assess the effectiveness of using muscular exercise to prevent or delay cachexia associated with alcohol and/or chemotherapy.

These research questions will be addressed using a mouse model in which implanted colon cancer cells readily lead to cachexia. Two different paradigms of alcohol consumption will be used to determine whether the cachectic effects differ if the patient stops drinking alcohol before tumor growth or chemotherapy treatment versus continuing to drink. Lastly, because exercise in the form of electrically stimulated muscle contraction has been shown to attenuate or prevent the loss of skeletal muscle caused by cancer, its efficacy in the presence of alcohol and/or chemotherapy treatment will be tested. The primary outcome of each experiment will be muscle size (i.e., cachexia development). Other variables will include measurement of molecular factors regulating muscle size, including those in muscle growth pathways and those involved in muscle breakdown. A variety of functional tests also will be used to determine the incidence and severity of fatigue as well as any changes in muscular strength or function.

This series of experiments will provide immediately translatable information to clinicians and patients about potential lifestyle changes that can be made to reduce their risk of cancer cachexia.

Scientifically, this work will provide novel foundational data pertaining to how alcohol increases skeletal muscle loss in the presence of cancer. It will lead to the future identification of therapeutic targets or therapies to mitigate cachexia risk in those who have consumed alcohol or continue to drink alcohol even after a cancer diagnosis.

Follow-on Funding: None at the time of reporting.

Collaborations: Jeong-Su Kim, PhD, Florida State University; Charles Lang, PhD, Penn State Hershey College of Medicine

Journals: None at the time of reporting.

Patents: None at the time of reporting.

4. #9BC04: Modeling Paths to Cancer Health Equity

Principal Investigator: George Rust, MD

Organization: Florida State University

Abstract of Proposed Research: While the quest to cure cancer continues, the greatest opportunity to dramatically reduce cancer deaths in the next decade will be to optimize utilization of existing innovations in effective screening and treatment across all segments of the population. Breast and colorectal cancers are two of the most screenable and treatable cancers, yet both still rank in the top five for cancer deaths. While death rates for each are declining in the U.S., the benefits of advances in early detection and treatment are disparate and not equitably shared. In fact, racial disparities in breast and colorectal cancer deaths have paradoxically widened despite myriad screening and treatment innovations. A major reason for this is that the benefits of these cancer innovations in early diagnosis and lifesaving treatments diffuse less quickly to disadvantaged segments of the population (racial-ethnic, socioeconomic, rural-urban and insurance sub-groups). These sub-group variations in uptake of new innovations are a preventable, yet a major contributor to health disparities. Additionally, racial-ethnic disparities in optimal application of these innovations varies at the local level from community to community. Racial-ethnic minority persons in one community might have easy access to screening tests but poor access to cutting-edge treatments, or vice versa.

Traditional prevention research and public health interventions have sought to reduce racial-ethnic disparities by testing the same intervention across many communities. Unfortunately, the intervention might be highly relevant to one community but not to another. For example, mobile mammography or cellphone text reminders for women to get screened might be very useful in a community that still has a low rate of breast cancer screening, such as the African-American community, but it might have no impact on a nearby community with high screening rates in all racial-ethnic groups. Missing, yet desperately needed, is a public health surveillance system and a strategic decision-support system that helps each community to understand where to target local interventions to achieve the most strategic impact on cancer outcomes.

This research program will create a method for using available data to define precisely the levels at which cancer disparities are being generated and amplified in each local community. The researchers will provide rapid throughput computer models of what the most strategic leverage points are in each local community to achieve the most optimal and equitable cancer outcomes possible. Application of these

models will provide a measure of “likely impact” for interventions at specific levels through common measures that define the relationship between screening rates, early diagnosis and survivorship, leading to reduced disparities. Ultimately, such mathematical and computer modeling will allow researchers to develop user-friendly web portals or even smartphone apps in which community health leaders can enter (from public health data sources) specific input parameters from their own community and manipulate variables to see what levels of interventions would be the most impactful in improving cancer outcomes and eliminating cancer disparities. Additionally, this would be a powerful tool for enhancing communications and community buy-in for these interventions, as well as assuring that investments made to improve cancer outcomes will have their intended impact.

Follow-on Funding: None at the time of reporting.

Collaborations: Charles Saunders, PhD, Co-Investigator, Florida State University College of Medicine; Debajyoti Sinha, PhD, Co-Investigator, Florida State University Department of Statistics; Maureen Sanderson, PhD, Collaborator, Meharry Medical College; Robert Levine, MD, Collaborator, Baylor College of Medicine; Henry Carretta, PhD, Co-Investigator, Florida State University College of Medicine; Dr. Jeffrey Harman, PhD, Co-Investigator, Florida State University College of Medicine; Penny Ralston, PhD, Co-Investigator, Florida State University College of Human Sciences and Director of the Center on Better Health & Life for Underserved Populations; and Lee Green, PhD, Collaborator, Florida Health Equity Research Institute (HERI).

Journals: None at the time of reporting.

Patents: None at the time of reporting.

5. Grant #9BC05: Metabolic Reprogramming to Improve Immunotherapy in Melanomas

Principal Investigator: Katarzyna A. Rejniak, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract of Proposed Research: Melanoma is the most aggressive among skin cancers. In the past decade, the number of newly diagnosed melanoma cases has increased by 53%. Florida ranks second in the nation for the highest rate of new melanoma cases. Melanomas are aggressive tumors resistant to radiation and chemotherapy. Tumor-specific T cells are present in the peripheral blood and tumors of melanoma patients. However, they fail to provoke tumor regression. Immunotherapies 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. Thus, additional immunosuppressive pathways may be active.

Dr. Rejniak and her team hypothesize that specific metabolic conditions (hypoxia and acidity) in the melanoma microenvironment play an immunosuppressive role. Their overarching hypothesis is that carefully planned manipulation of the tumor microenvironment can result in improved melanoma response to immunotherapy. Their research methods include computational simulations informed by experimental data to direct experiments with defined T cells in a murine model of melanoma. They employed integrated experimental and computational techniques to predict the most optimal immunotherapeutic interventions at physiologically relevant potential hydrogen (pH) (Aim No. 1) and oxygen (O2) (Aim No. 2) that will lead to anti-tumor immunity.

In the first quarter of the funding period, the team pursued research in both aims and concentrated on the development of the mathematical model, testing model simulations and collecting experimental in vitro data for model calibration. Changes in the properties of T cells were characterized when exposed to various levels of acidic and hypoxic conditions in vitro. These results showed that the T cell-produced

interferon (IFN)-gamma was decreased in both severe acidic and severe hypoxic conditions indicating that T cell activity is decreased with increased hypoxia and increased acidity.

The phase of in silico model calibration has been completed with the morphology and metabolism of in vivo B16 melanoma. This computational model was used for investigating the interplay between the IFN-gamma secreted by the immune cells and the tumor tissue hypoxia. Computational experiments were conducted to test how IFN-gamma distribution depends on the number of injected T cells and T cells motility. These model simulations showed that the doubling of the number of injected T cells results in a better distribution of IFN-gamma than the doubling of T cells migration speed within the tissue.

The research team has been involved in translational research in the immunotherapy of cancer for many years. It will continue efforts to develop an optimal treatment regimen for the regression of established melanoma. These murine tumor studies and in silico models are intended to improve the design and execution of clinical trials in melanoma patients. Moffitt Cancer Center has devoted strong efforts toward melanoma research and treatment within the Donald A. Adam Comprehensive Melanoma and Skin Cancer Research Center of Excellence. Therefore, the results of these studies can lead to clinical trials at the Moffitt clinic.

Follow-on Funding: National Institutes of Health (NIH); *Modulating the Tumor Microenvironment to Improve Immunotherapy in Melanoma*; Shari Pilon-Thomas, PhD, Katarzyna A. Rejniak, PhD; $3,094,374 (Pending).

Collaborations: This research is in collaboration with Tamas Kis, an undergraduate student at Vanderbilt University, who participated in Moffitt's SPARK**,** the Summer Program for the Advancement of Research Knowledge and conducted research in Dr. Rejniak's lab. 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: Chamseddine IM, Rejniak KA. Hybrid modeling frameworks of tumor development and treatment. *Wiley Interdiscip Rev Syst Biol Med* 2019 Jul 17:e1461. doi: 10.1002/wsbm.1461. PMCID:31313504.

Patents: None at the time of reporting.

6. 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

Abstract of Proposed Research: The decreased anti-tumor immunity present in most patients and experimental animals with cancer represents a major limitation in the successful development of different forms of cancer therapy. Multiple pro-inflammatory mediators produced in the tumor microenvironment induce the accumulation of myeloid-derived suppressor cells (MDSCs). These are a heterogeneous population of immature cells that potently block protective anti-tumor immunity and have emerged as a major obstacle to new cancer treatments, including immunotherapy. Despite MDSCs' undeniable relevance in tumor-induced immune suppression, there are no current approaches to effectively block their immunosuppressive activity in patients with cancer. Thus, novel therapeutic strategies to inhibit MDSCs are urgently needed.

With this bridge funding, the research team aimed to identify the mechanisms by which the expression of the Notch ligands, Jagged1-2, in tumor-bearing hosts functionally drives MDSCs' functionality. The

overall hypothesis is that the expression of Jagged1-2 in cancer cells plays a central role in the suppression of protective T cell immunity in tumors.

The research team proposes 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 MDSCs' immunosuppression in tumors; and 3) investigate the role of the stimulator of interferon genes (STING)-associated Type I interferon production as the driver signal mediating the immunogenic effects induced by anti-Jagged1-2 therapy in tumor-bearing hosts.

During this initial period, the team aimed to elucidate the effect of the expression of Jagged 1 and/or 2 in tumor cells in the immunosuppressive activity of tumor-MDSCs. Thus, a model was developed for the elimination of Jagged 1 ($Jag1^{-/-}$) and/or Jagged 2 ($Jag2^{-/-}$) in lung tumor cell line Lewis lung carcinoma (LLC).

Research has advanced as follows:

- Successful development of the $Jag1^{-/-}$ and/or Jagged 2 ($Jag2^{-/-}$) LLC cells. Experiments completed after transferring these cell lines into mice showed that elimination of Jagged 1 and 2 dramatically blocked tumor growth.
- Isolation of MDSCs from mice bearing $Jag1/2^{-/-}$ displayed a significant decreased ability to inhibit the proliferation of T cells, which correlated with altered expression of the MDSCs-associated immunosuppressive factors Arginase and Nos2.
- Elimination of Jagged 1/2 in tumor cells did not affect the expansion or distribution of MDSCs subsets in tumors, indicating that elimination of Jagged on tumors affected MDSCs' immunosuppressive activity, rather than infiltration, proliferation or MDSCs differentiation.
- Detection of elevated accumulation of polyfunctional $CD8^+$ (cytotoxic) T cells producing anti-tumor molecules interferon-gamma (IFNγ) and tumor necrosis factor-alpha (TNFα) in tumors from mice bearing $Jag1/2^{-/-}$ tumors. Also, the team observed enhanced accumulation of myeloid cells expressing major histocompatibility complex (MHC) class II and *cluster of differentiation 11c (*CD11c), rather than type 1 dendritic cells (DC1) cells, which could suggest the accumulation of myeloid-DC populations.

In summary, the researchers believe that their accomplishments, together with the contribution of the covered key personnel and the strong environment at Moffitt Cancer Center, have favorably impacted the completion of the proposal. Continuation of this research is expected to have an impact on the health of Floridians by elucidating primary events occurring in tumors, such as lung carcinoma and melanoma, and by improving the therapies against those 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.

7. 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

Abstract of Proposed Research: Lung cancer is the leading cause of cancer-related death. Mutations in the nuclear factor erythroid 2/Kelch-like ECH-associated protein 1 (NRF2/KEAP1) circuit are among the most common mutations in lung cancer, are suggested to cause chemoresistance/radioresistance and are enriched in tumors that fail to respond to targeted therapy.

Research project staff will evaluate 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. This research project has two aims: *1*) To target NRF2-regulated metabolism for cancer therapy and 2) To relate NRF2/KEAP1 mutations and pathway activation with therapeutic response.

The goal of the first aim is 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 will evaluate two approaches: 1) To block the metabolism of a key nutrient, cysteine; and 2) to exploit an NRF2-regulated enzyme, NQO1, to selectively kill mutant tumor cells.

Researchers have optimized the dosing strategy for mouse experiments for approach No. 2 and have started the preclinical evaluation of this approach in lung tumor models. They have also generated additional feasibility data for approach No. 1 — which suggests that lung cancer cells will be universally sensitive to this approach — and are examining whether any resistance mechanisms might be expected. Preclinical studies will begin soon.

The goal of the second 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.

The progress is outlined below.

<u>Chemotherapy response</u>

For chemotherapy cohort 1: 870 NSCLC patients treated with platinum-based chemotherapy (cisplatin, carboplatin) were identified. Cancer characteristics such as primary site and histology were queried from Moffitt's Cancer Registry. These patients also have survival information, such as vital status, date of last contact, death and causes of death, if available. The next step for this cohort includes querying radiation therapy data (if available) and estimating the sample size for which tissue will be requested for NRF2/KEAP1 sequencing.

For chemotherapy cohort No. 2: 197 patients were identified from a Moffitt-led study that assessed whether chemotherapy would improve survival in patients with advanced NSCLC. A query of pre-treatment tissue availability for this cohort is in progress.

<u>Radiation response</u>

Patient cohort and tissues must be identified at Ohio State University. RNA has been isolated and the association between NRF2 activation (a surrogate for KEAP1/NRF2 mutation) and radiation response is in progress.

<u>Immunotherapy response</u>

A cohort of 181 NSCLC patients treated with immunotherapy (nivolumab, pembrolizumab, durvalumab, and atezolizumab) with pretreatment tissue available were identified. Further data query to extract information on cancer characteristics, treatment history and survival data is in progress.

Follow-on Funding: None at the time of reporting.

Collaborations: Dr. Terrence Williams, Department of Radiation Oncology, Ohio State University, Columbus, Ohio. 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.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

8. 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

Abstract of Proposed Research: 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. HCT, however, only cures some of the patients with AML. 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. Furthermore, survival without leukemia recurrence drops to only 25% at one year after HCT. Immunotherapy with donor lymphocytes administered after HCT has increased cures and prolonged survival of patients with residual AML detected at the time of HCT. However, some donor lymphocytes, called aβ T cells, can attack the patient's 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 gamma delta T cells (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.

The research team has engineered artificial antigen presenting cells that activate γδ T cells in the laboratory and achieved up to a thousand-fold expansion of healthy donor blood γδ T cells. A hypothesis is that donor γδ T-cell infusion in patients with post-chemotherapy residual AML can prevent leukemia recurrence after HCT without causing GVHD and improve survival without leukemia recurrence from 25% to 50%.

The purpose of this project is to:

1) Determine the safety and effectiveness of artificial antigen presenting cells-expanded donor γδ T cells infused after HCT for treatment of patients with evidence of 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.

Infusion of donor γδ T cells expanded in the laboratory for the prevention of leukemia recurrence after HCT is highly innovative.

2) Study the leukemia cell-killing activity of chimeric antigen receptor (CAR)-engineered γδ T cells. The researchers intend to explore whether the potency of γδ T cells can be enhanced by engineering them in the research team's laboratory with a CAR specific for AML cells.

Hypothesis: γδ T cells engineered with a synthetic receptor developed in the research team's laboratory will be more effective in killing AML leukemia cells than non-engineered γδ T cells or CAR aβ T cells.

These experiments are innovative and will direct future development of synthetic CAR γδ T cells for immunotherapy of AML.

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 #9BC09: Development of Novel TAF1 Inhibitors as Cancer Therapeutics

Principal Investigator: Ernst Schonbrunn, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract of Proposed Research: The bromodomain-containing protein TAF1 (thioredoxin binding protein [TBP]-associated factor 1) 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 a 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 research staff discovered that a clinical inhibitor of the protein kinase ataxia telangiectasia and Rad3-related (ATR) also selectively inhibits the second bromodomain of TAF1. This is the first identified kinase inhibitor that targets bromodomains outside the bromodomain and extra-terminal motif (*BET*) family. The team determined high resolution co-crystal structures of TAF1 liganded with this inhibitor and close analogues. This knowledge 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 phosphoinositide 3-kinases (PI3K)-related kinases. The team's preliminary studies in lung and colon cancer cell lines established that TAF1 inhibitors activate p53 and DNA damage response and induce p21 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. The 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 first year involves cell biological, chemical and structural studies. Cellular TAF1 interaction with wild-type or mutant p53 was not influenced by inhibitors, suggesting that the

bromodomains of TAF1 are not involved in this protein-protein interaction. However, TAF1 showed increased expression levels in the presence of inhibitors suggesting that bromodomain inhibitors stabilize TAF1 against protein turnover. A phospho-specific antibody of p53 (T55) has been generated to investigate the role of TAF1 in regulating T55 phosphorylation and p53 DNA binding. Five new analogues of the parent inhibitor AZD6738 were synthesized and subjected to structure-activity relationship studies to assess binding potential toward TAF1 by biochemical and structural methods. While binding affinity was not improved over the parent compound, co-crystal structures of TAF1 were obtained with two analogues. This new structural information is currently being used for the rational design of new analogues with improved binding potential for TAF1.

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 #9BC10: Role of Intercellular Adhesion Molecules 1 (ICAM1) in Development and Progression of Pancreatic Cancer

Principal Investigator: Peter Storz, PhD

Organization: Mayo Clinic

Abstract of Proposed Research: According to the National Institutes of Health (NIH) State Cancer Profiles, the average annual incidents of pancreatic cancer in Florida are rising. In 2018, 3,800 new cases of pancreatic cancer were expected, with approximately equal numbers for both sexes.

Pancreatic cancer carries a dismal prognosis, because it usually is detected at a stage where it already has metastasized. There are limited therapeutic options apart from surgery, and due to early metastasis, even patients with resected tumors have a survival rate of less than a year. Understanding the mechanisms that promote development and progression of pancreatic cancer in order to identify novel methods of early detection and intervention is the greatest hope for preventing and treating this cancer.

This grant was bridge funding for an R01 application submitted to the NIH. The project is to define critical mechanisms involved in pancreatic carcinogenesis. It was designed to assess novel candidate biomarkers that may aid in the detection of precancerous lesions at an early stage but also indicate treatment response. Moreover, the researchers will develop and test novel immunotherapy approaches, either alone or in combination with standard of care chemotherapy. Overall, this project will lead to the preclinical data needed for the development of novel clinical trials to better the outcome for patients with advanced cancer.

With this bridge funding, the staff has generated scientific tools and started performing the experiments needed to successfully obtain federal funding for the project.

Follow-on Funding: National Institutes of Health (NIH); *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.

11. Grant #9BC11: Identify the Mechanisms of LM2-Mediated Inhibitor of Homologous Recombination and Establish PARP-Targeted Synthetic Lethality as a New Therapy

Principal Investigator: Izidore Lossos, MD

Organization: University of Miami

Abstract of Proposed Research: Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin's lymphoma (NHL), with about 25,000 new cases yearly. Despite marked improvement in therapy, about half of these patients succumb to their disease. Therefore, there is a strong need for new therapeutic approaches to improve DLBCL patients' survival. The research team showed that in DLBCL cells the LIM domain-only 2 (LMO2) protein inhibits DNA double-strand break (DSB) repair via homologous recombination (HR), resulting in HR-dysfunction. This HR-dysfunction phenocopies BRCA1/2 mutations in breast, ovarian and castration-resistant prostate cancers. Accordingly, the team showed that LMO2 predisposes DLBCL cells to synthetic lethality upon treatment with poly(adenosine diphosphate-ribose) polymerase 1 and 2 (PARP1/2) inhibitors (PARPi). The long-term goal is to demonstrate that PARPi activity may improve the outcome of patients with LMO2 expressing DLBCL.

The overall objectives of this proposal are to determine the mechanisms by which LMO2 inhibits the repair of DNA breaks via HR and whether LMO2 expression levels can be exploited as a biomarker for sensitivity of DLBCL to PARP1/2 inhibitors. The central hypothesis is that inhibition of DNA repair via HR induced by LMO2 will sensitize DLBCL tumors to PARP1/2 inhibitors. The rationale for this project is that deficiency in HR and failure to repair DSBs produced during replication can lead to genomic instability and/or cell death. Indeed, PARP1/2 inhibitors that cause the accumulation of toxic DSBs during replication, had been exploited for the treatment of HR-deficient solid tumors. Our preliminary data showed that in DLBCL cells LMO2 inhibits the HR pathway.

Thus, the researchers propose that inhibition of HR by LMO2 will sensitize DLBCL tumors to PARP1/2 inhibitors. In order to test the central hypothesis and determine the mechanisms by which LMO2 controls DNA repair, the team also proposes three specific aims: 1) Identify the mechanism(s) of LMO2-mediated inhibition of HR in DLBCL; 2) Determine how LMO2 affects immunoglobulin class switch recombination in normal B cells; and 3) Demonstrate that DLBCL expressing LMO2 are sensitive to PARP1/2 inhibition. The proposed research is innovative because it represents a substantive departure from the current status quo by demonstrating that expression of LMO2 protein predicts therapeutic activity of PARP1/2 inhibitors in DLBCL and represents an effective new therapeutic strategy that will broaden the existing arsenal against this lymphoma. The research proposed is significant because it is expected to provide strong scientific justification for the development of a novel therapeutic approach for DLBCL based in PARP1/2 inhibitors that could potentially change the current treatment of DLBCL patients and improve their outcome.

These studies will generate evidence for the rationale to use PARPi alone and in combination with DNA-damaging chemotherapy for clinical trials in DLBCL.

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 #9BC12: Development of Small Molecule Inhibitors of Wnt/Beta-Catenin Transcriptional Activation

Principal Investigator: Anthony Capobianco, PhD

Organization: University of Miami

Abstract of Proposed Research: Deregulation of the *wingless/int-1* class (Wnt) /β-catenin signaling pathway has been demonstrated to play a role in tumorigenesis, as evidenced by its involvement in multiple malignant tumors in humans. This pathway is also implicated in the maintenance and survival of cancer stem cells, which may confer resistance to chemotherapy. The important role played by Wnt/β-catenin signaling in cancer makes it an exceedingly attractive target for cancer therapeutics. However, the full range of potential targets in the pathway has been under-explored. To date, there are no small molecule inhibitors that successfully target the intracellular Wnt/β-catenin signaling pathway. Once β-catenin translocates to the nucleus, it is involved in the formation of a core transcriptional activation complex, where it binds to T-cell factor/lymphoid enhancer factor (TCF/LEF), B-cell lymphoma 9-like protein (BCL9)/BCL9-2 and other cofactors, which then recruit additional members of the transcriptional machinery. This β-catenin nuclear complex initiates and maintains transcriptional activation of Wnt target genes. The overarching hypothesis of this proposal is that compounds that prevent the formation of the β-catenin nuclear complex by targeting β-catenin and BCL9/BCL9-2 interface would be potent inhibitors of the Wnt/β-catenin pathway. The researchers have used a combination of computational and biochemical studies and identified a lead compound that inhibits Wnt/β-catenin signaling. Therefore, the overall goal of this project is to optimize the scaffold of this lead compound to identify clinical candidates that inhibit assembly of the β-catenin nuclear complex in order to develop novel potent drug-like small molecule inhibitors of Wnt/β-catenin mediated transcription. To this end, the team will use an innovative approach that combines current state-of-the-art computational, biochemical and biophysical techniques. Successful completion of this study will fulfill an unmet need in terms of therapeutic agents targeting the Wnt/β-catenin signaling pathway that specifically target the β-catenin nuclear complex, therefore providing specific inhibition of Wnt/β-catenin transcriptional activation complex, which could complement and/or offer an alternative to current therapeutic approaches. The team will achieve the goals of this proposal through the following specific aims: 1) Lead optimization through structure-activity relationship studies; 2) Biochemical and biological assessment of lead analogs; and 3) Preclinical evaluation of efficacy of lead clinical candidates.

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

Principal Investigator: Kerry L. Burnstein, PhD

Organization: University of Miami

Abstract of Proposed Research: In Florida in 2019, 11,860 men will be diagnosed with prostate cancer

(PC) and 2,290 will die of this malignancy, per American Cancer Society estimates. Advanced PC is particularly challenging to treat, because tumors almost always develop "resistance" to drugs, leading to incurable cancer growth. Tumors acquire resistance by strategies that vary between patients and even between tumors in the same patient. Race and ethnicity also contribute to differences in PC tumors and their response to drugs. Thus, no single therapeutic regimen will treat all aggressive PC. Incurable stages of PC require a precision medicine approach: treatments tailored specifically to features of individual patients' tumors. Fortunately, researchers have huge amounts of molecular, genetic and clinical information on PC from a broad variety of patients. With advanced computational methods, researchers are beginning to identify distinct gene "signatures" in drug-resistant tumors. The challenge is to exploit this "big data" efficiently and rationally to identify and prioritize new drugs. Such data-driven, signature-based approaches already have led to "drug repurposing," in which drugs for certain diseases are prescribed for different diseases including PC. Also, combining two drugs has proven highly promising for prostate and other cancers, often yielding clinical responses greater than the sum of the individual drugs (termed “synergy”).

This study leverages the complementary expertise of two investigators: a PC researcher with a robust record of identifying and testing new experimental PC therapies and a chemist/data scientist pioneering the use of big data to identify new drugs and drug combinations (as well as entirely novel methods) to block cancer growth.

This study integrates collections of big data, including gene signatures specific to and representative of a large variety of prostate tumors, with the known responses of over 50 human cancer cells (including PC) to over 1,500 Food and Drug Administration (FDA)-approved drugs in clinical trials plus thousands of drug-like molecules. A computational algorithm will identify drugs with a known effect on PC-specific gene signatures that are therefore predicted to block growth of tumors with distinct features. A novel computational platform (developed by one of the investigators) evaluates tens of millions of drug combinations to predict the precise combinations that will block growth of different PC tumors. The highest-ranked predicted drug combinations will be tested in PC cell lines and then in mice bearing different human tumors (mimicking different stages of PC in men). Successful treatment of cancer requires initiating the right therapy at the most opportune time. Therefore, this study will improve PC treatment outcomes by identifying patient-specific targeted therapies customized to the exact tumor type and the exact stage of tumor development.

The following progress was made in the first quarter: Patient sample data were acquired and processed, yielding 501 PC samples and 52 non-cancer prostate samples. PC-specific gene-signatures were identified for computational screening against the array of drugs, drug-like compounds and drug combinations. For initial drug testing, cell lines from PC, advanced PC and non-cancer prostate tissue were prepared and validated for testing with new drugs and drug combinations.

Follow-on Funding: None at the time of reporting.

Collaborations: Stephan Schurer, 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. Rimpi 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. Julia Martinez to guide drug synergy analyses methodologies.

Rimpi Khurana, PhD, University of Miami (post-doctorate) performs RNA-sequencing (RNAseq)

processing and analytics including gene set enrichment and network analysis. She executes computational algorithms based on patient RNAseq data to robustly identify gene co-expression networks. She 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-doctorate) performs all cell-based studies and conducts tumor analysis.

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

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: HongYuan (Rays) Jiang, PhD

Organization: University of South Florida

Abstract of Proposed Research: Researchers plan to use single-cell omics expertise to construct the first single-cell cancer iron metabolic map as well as uncover the first cancer microenvironment of iron addiction. Iron is essential for cell growth and replication; aggressive or metastasized cancers often require large amounts of iron, called iron addiction. Iron metabolism is tightly linked to heme production, as iron exists mostly in the form of heme, enclosed within an organic protoporphyrin ring in all mammals. Particularly, it is hypothesized that iron addiction is inextricably linked with heme overdrive, the heightened embryonic-like heme metabolism in cancers. Significantly, pancreatic, lung and skin cancers as well as leukemias are iron addicted as shown by clustered regularly interspaced short palindromic repeats (CRISPR) studies. Research staffers propose to uncover the critical metabolic pathways underlying cancer iron addiction.

During this initial preparation phase of the project, related experimental permits are being prepared. Importantly, a significant part of this project is related to data generation and processing. The University of South Florida computational capacity is being utilized to set up and optimize the single-cell metabolic studies by using pilot data, as an example.

The following studies will be performed: 1) Single-cell RNA sequencing (scRNA-seq), and 2) single-cell metabolic network constructions. Obtaining these results will help to uncover the origin of acute myeloid leukemia, or AML, malignancy, both in gene expression and enzyme protein quantity, and will be tracked with two complementary cutting-edge technologies: scRNA-seq and mass cytometry. During the next phase, the researchers are discovering the cancer niche supporting iron addiction and focusing on disruption of the aberrant iron and heme trafficking of the cancer micro-environment. The final goal of the project is to use forward genetics to uncover iron-addicted cancer vulnerability.

Follow-on Funding: None at the time of reporting.

Collaborations: HongYuan (Rays) Jiang, PhD, Corresponding Principal Investigator, College of Public Health, University of South Florida; Gloria Ferreria, PhD, Principal Investigator, Morsani College of Medicine, University of South Florida

Journals: None at the time of reporting.

Patents: None at the time of reporting.

15. 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

Abstract of Proposed Research: Melanoma is the deadliest form of skin cancer with frequent activation of the reticular activating system/rapidly accelerated fibrosarcoma/mitogen-activated protein kinase, or RAS/RAF/MAPK, signaling pathway. Researchers recently reported that tyrosine protein phosphatase non-receptor type 11 (PTPN11) plays tumor promoting roles in *proto-oncogene B-RAF* wild-type melanoma by activating RAS signaling pathway and is frequently activated in melanoma patients. This study was presented as a poster at the American Association for Cancer Research's (AACR) annual meeting in April 2019 and as an invited talk at the AACR Special Conference on Melanoma: From Biology to Target in Houston (January 2019); in the departmental seminar series at the University of Houston (January 2019); and at the 13th KWiSE (Korean-American Women in Science and Engineering) West Coast Annual Conference (La Mirada, California, May 2019).

This proposed study aims to: 1) Identify downstream effectors of PTPN11 and the resistance mechanisms to PTPN11 inhibition; 2) Develop a rational combined therapy based on PTPN11 function in order to enhance the response rates in melanoma patients; and 3) Assess the effect of PTPN11 inhibition on tumor and immune cells utilizing an activated PTPN11 mouse melanoma model the research team generated. Recently, a new class of drugs targeting PTPN11 has been developed and is currently being tested in clinical setting for safety. A preliminary study showed that inhibition of PTPN1 caused regression of tumors, which was associated with decreased growth and increased death of melanoma cells as well as increased recruitment of immune cells into the tumors. It also was observed the importance of glycogen synthase kinase, or GSK3/β-Catenin/Cyclin D1 signaling pathway as a downstream of PTPN11 function.

This proposed study will allow one to understand the molecular mechanisms underlying the response of melanoma to PTPN11 inhibition and will identify a better way to predict a patient's likelihood of responding to PTPN11 inhibition allowing for novel therapeutic opportunities for melanoma patients. In addition, this study will validate PTPN11 as a novel therapeutic target in melanoma, providing a path to the clinic.

Follow-on Funding: National Institutes of Health (NIH)/National Cancer Institute; *Developing Strategies Targeting BRAF Wild-Type Melanoma Based on PTPN11 Inhibition*; Minjung Kim, PhD; $1,805,883 (requested).

Collaborations: Researchers are collaborating with Dr. Sungjune Kim at the Departments of Immunology and Radiology at Moffitt Cancer Center to analyze the effects of PTPN11 inhibition on immunological profiles.

Journals: Hill KS, Roberts ER, Wang X, Marin E, Park TD, Son S, Ren Y, Fang B, Yoder S, Kim S, Wan L, Sarnaik AA, Koomen JM, Messina JL, Teer JK, Kim Y, Wu J, Chalfant CE, Kim M. PTPN11 plays oncogenic roles and is a therapeutic target for BRAF wild-type melanomas. *Mol Cancer Res* 2019;17(2):583-593. doi: 10.1158/1541-7786.MCR-18-0777. PMID: 30355677. PMCID: PMC6386183.

Patents: None at the time of reporting

APPENDIX B

FISCAL YEAR 2018-2019 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	Copland, John	$ 815,283	$ 286,451.00	$ 528,832.00	5/01/2018	4/30/2021	No	No	No
8BC02	H. Lee Moffitt Cancer Center	Ruffell, Brian	$ 815,289	$ 271,760.00	$ 543,529.00	4/20/2018	3/31/2021	No	No	No
8BC03	H. Lee Moffitt Cancer Center	Smalley, Keiran	$ 815,283	$ 271,760.00	$ 543,523.00	4/02/2018	3/31/2021	No	Yes	Yes
8BC04	H. Lee Moffitt Cancer Center	Gillies, Robert	$ 815,283	$ 271,760.00	$ 543,523.00	3/21/2018	3/31/2021	No	Yes	No
8BC05	University of Central Florida	Phanstiel, Otto	$ 815,283	$ 271,761.00	$ 543,522.00	5/30/2018	3/31/2021	Yes	No	Pending*
8BC06	University of Miami	Hudson, Barry	$ 815,283	$ 271,760.00	$ 543,523.00	4/20/2018	3/31/2021	Yes	No	Yes
8BC07	University of Miami	Saluja, Ashok	$ 815,282	$ 271,760.00	$ 543,522.00	4/04/2018	3/31/2021	No	No	No
8BC09	University of Miami	Wieder, Eric	$ 1,358,805	$ 721,651.00	$ 637,154.00	6/08/2018	3/31/2021	No	No	No
8BC10	University of Miami	Dhar, Shanta	$ 815,283	$ 271,761.00	$ 543,522.00	4/11/2018	3/31/2021	No	No	No
8BC12	Florida State University	Deng, Wu-Min	$ 815,283	$ 238,821.50	$ 576,461.50	6/12/2018	3/31/2021	No	No	No

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

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

Principal Investigator: John A. Copeland, PhD

Organization: Mayo Clinic

1. Grant Progress Report: The research team has examined the effect of *s*treptomyces subtilisin inhibitor-4 *(*SSI-4), anti-programmed cell death protein 1 (PD1), or a combination of the two, tested against a mouse colorectal cancer cell line (MC38) implanted subcutaneous. Tumor volume is plotted over time and shows single agent activity of both treatments. There does not appear to be a combinatorial effect on tumor volume from this experiment.

The research team has also explored the effect of SSI-4 combined with anti-PD1 in a Renca renal immune competent mouse model. The team observed single agent activity compared to placebo control with no combination anti-tumor activity. Interestingly, if separated between male and female groups, tumors grown in females appear to show resistance while males do not. Some combination therapy time points are statistically significant. Thus, sex makes a difference in response and not all tumor types respond to treatment.

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

Principal Investigator: Brian Ruffell, PhD

Organization: H. Lee Moffitt Cancer Center & Research Institute

Grant Progress Report: The researchers have previously established that immune checkpoint blockade (ICB) targeting the molecule T cell immunoglobulin and mucin domain containing protein 3 (TIM-3) leads to activation of a population of immune cells responsible for regulating the immune response (i.e., dendritic cells). As a result, TIM-3 blockade promotes the anti-tumor function of cytotoxic T lymphocytes and improves response to chemotherapy in murine models of breast cancer. The purpose of this grant is to determine the mechanism by which this occurs.

Researchers have found that TIM-3 negatively regulates the uptake of extracellular DNA by dendritic cells. Blocking TIM-3 therefore leads to DNA uptake, activation of the stimulatory of interferon genes (STING) pathway, expression of type I interferons and finally expression of the chemokines CXCL9 and CXCL10. The research team has also established that a similar mechanism of action applies to human dendritic cells, with both DNA uptake and chemokine expression observed following TIM-3 blockade. These findings suggest that blocking antibodies against TIM-3 may be a viable therapeutic strategy to improve response to chemotherapy in breast cancer patients, and that future studies to optimize combinatorial strategies are warranted.

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 #8BC03: Defining and Therapeutically Targeting HDAC8-Driven Reprogramming in Melanoma Brain

Principal Investigator: Keiran Smalley, PhD

Organization: H. Lee Moffitt Cancer Center & 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 about 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. This represents a major knowledge gap that limits the ability to deliver long-term therapeutic responses to melanoma patients.

In preliminary studies, the research team uncovered a novel gene expression program regulated through an enzyme called histone deacetylase 8 (HDAC8) 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 determine how this cellular state permits the melanoma cells to form new tumors in the brain. Further studies will address whether new therapeutic strategies can be developed in which HDAC8 is targeted in combination with established melanoma therapies such as targeted therapy (*B-Raf murine sarcoma viral oncogene homolog* B1 [BRAF] inhibitors) and immunotherapies. This project is expected to bring important new insights into the biology of brain metastases, allowing new therapies to be developed.

In the first year of funding, researchers have made major progress toward achieving these goals. First, the team has identified a novel mechanism by which HDAC8 regulates the metastatic program in melanoma cells. The experiments showed that HDAC8 alters the acetylation of a key transcription factor called c-JUN that is required for driving metastatic behavior. The team demonstrated for the first time that HDAC8 directly acetylates c-JUN at Lysine 273, and that this, in turn, switches on a critical gene expression program in the melanoma cells that makes them highly aggressive. This cellular program was also conserved in normal skin melanocytes (the pigment producing cells of the skin) and likely constitutes a stress response that melanocytes use to adapt to sun exposure. This work was published in the prestigious journal *Cancer Research*.

Researchers are currently working with colleagues in the Drug Discovery Department at Moffitt Cancer Center to develop new drugs that inhibit HDAC8. They believe that such a drug could be useful to both prevent the development of melanoma metastases and overcome resistance to commonly used drugs in melanoma.

At the same time, the team has also been exploring how this HDAC8-driven cellular program contributes to the development of melanoma metastases in mice. Researchers have made the important observation that HDAC8 alters expression of an adhesion protein called EphA2 that allows the melanoma cells to stick to the surface of blood vessels and then migrate into other organs such as the brain. It is currently testing whether inhibition of EphA2 signaling prevents the melanoma cells from being able to metastasize to the brain.

Follow-on Funding: Melanoma Research Foundation; *Defining Drivers of Melanoma Metastasis*; Keiran Smalley, PhD; $100,000.

Collaborations: This project is a 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 clustered regularly interspaced short palindromic repeats (CRISPR) screening. Dr. Licht and Dr. Smalley's labs have initiated regular Zoom video conferencing meetings that are held every three weeks. These provide a forum to share data, discuss progress with the project and troubleshoot any issues that arise. Dr. Licht and Dr. Smalley met in person in June 2019 in Washington (they sit on the same National Institutes of Health study section) to discuss progress on the grant. 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, a staff scientist at Moffitt Cancer Center; Dr. Amin Sobh, a post-doctoral University of Florida student; and Dr. Richard Bennett, a research assistant professor at the University of Florida.

Journals: Emmons MF, Flores F, Sharma R., Thapa RS, Messina JL, Becker JC, Schadendorf D, Seto E, Sondak VK, Koomen JM, Chen YA, Lau EK, Wan L, Licht JD, Smalley KS. HDAC8 regulates a stress response pathway in melanoma that mediates escape from BRAF inhibitor therapy. *Cancer Res* 2019;79:2947-2961. doi: 10.1158/0008-5472.

Patents: None at the time of reporting.

4. 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 highly acidic extracellular microenvironment. Adaptation to acidic conditions is a prerequisite for tumor cells to survive, thrive and outcompete the stroma into which they invade. Acid adaptation has been associated with chronic activation of autophagy and redistribution of the lysosomal proteins to the plasma membrane. In addition to these survival mechanisms, tumor cells under acidic conditions accumulate cytoplasmic lipid droplets (adiposomes) — dynamic organelles that store neutral lipids surrounded by a shell of proteins such as Perilipin-2 (PLIN2) and a phospholipid monolayer.

High expression of PLIN2 was observed to be strongly associated with poor overall survival in breast cancer patients. In vitro, breast cancer cells rapidly and robustly accumulated adiposomes when grown in acidic media. The acid-induced lipogenic phenotype persisted even when the cells were grown in delipidated serum and was inhibited by fatty acid synthesis inhibitors. This indicates that the source of lipids is de novo and endogenous. Further, these inhibitors were selectively cytotoxic under acidic conditions, indicating that adiposomogenesis is a survival mechanism. 13C isotopomer analysis showed a major shift in glucose metabolism to the pentose phosphate pathway (PPP), providing the nicotinamide adenine dinucleotide phosphate (NADPH) necessary for de novo lipogenesis. Furthermore, G6PD, the rate-limiting enzyme in the PPP, was transcriptionally upregulated in cells grown under acidic media. When cells were treated with inhibitors of PPP, either using 6PGD inhibitor Physcion, 6-aminonicotinamide, or the G6PD inhibitor polydatin, adiposome accumulation under low pH was significantly inhibited. Cell viability was also reduced, indicating the involvement of PPP in acid-induced adiposomogenesis.

Since lowering extracellular pH does not affect the intracellular pH, the research team tested the hypothesis that the acid signal was mediated by one (or more) acid sensing G-protein coupled receptors.

Both *ovarian cancer G protein-coupled receptor 1* (OGR1) and T cell death-associated gene 8 (TDAG8) were strongly expressed in the team's systems. Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9)-mediated depletion of these receptors showed that TDAG8 knockout (KO) had no effect, but that KO of OGR1 abrogated adiposome accumulation under acidic conditions. OGR1 is coupled with guanine nucleotide-binding protein Gq/11, and upon activation, can trigger calcium ions (Ca2+) release from intracellular Ca2+ stores through phospholipase C (PL-C) and stimulate formation of the second messenger, inositol trisphosphate (IP3). PLC inhibition at low pH using U73122 resulted in attenuation of adiposome accumulation. Further, OGR1 knockout cells were defective in acid-induced autophagy. Hence, accumulation of adiposomes is a highly regulated process related to storing autophagic products and appears to be important in cell survival under acid stress.

To identify the carbon source for lipids accumulated in adiposomes, the research team incubated cells that were undergoing acid-induced adiposomogenesis with different 13C labeled carbon sources: glucose, acetate, lactate and glutamine. The team then measured the accumulation of 13C into adiposomal lipids using mass spectrometry. Since ketogenic amino acids derived from acid-induced autophagy could be a potential carbon source for the acyl chains in adiposomal lipids, research staff labeled cells with 3-13C leucine to steady state. Cells were treated with acidic culture media for 72 hours and allowed to form adiposomes, and these were extracted and analyzed by mass spectrometry. Results indicate that leucine is a major source of carbon in adiposomal lipids.

Follow-on Funding: None at the time of reporting.

Collaborations: The University of Florida, Department of Pathology, Immunology and Laboratory Medicine in Gainesville, has been involved in this research project through analytical measurement of lipid species using ultra-high-pressure liquid chromatography (UHPLC) coupled with high resolution mass spectrometry. Currently, Dr. Timothy Garrett (the Southeast Center for Integrated Metabolomics (SECIM, University of Florida) is working on this project and has recruited a post-doctoral fellow, Dr. Iqbal Mahmud, to continue more detailed mass spectrometry analysis to identify the carbon source of adiposomal lipids. Dr. Garrett is currently working on analytical improvement of methodologies and employing new software tools to explore the isotopic flux of precursors into intact lipids species. He is also developing new tools for data mining of isotopes in lipids. Samples have also been provided to the Nuclear Magnetic Resonance facility for additional characterization of lipid labeling.

Journals: Pillai SR, Damaghi M, Marunaka Y, Spugnini EP, Fais S, Gillies RJ. Causes, consequences, and therapy of tumors acidosis. *Cancer Metastasis Rev* 2019 Jun;38(1-2):205-222. doi: 10.1007/s10555-019-09792-7. PMID: 30911978. PMCID: PMC6625890 [Available on 2020-06-01].

Patents: None at the time of reporting.

5. 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: The purpose of this grant Is to develop new medicines to treat pancreatic cancers which currently have a five-year survival rate of less than 8%. The context of this proposal is to target a metabolic addiction of these tumors, which drive their growth through upregulated polyamine metabolism. Polyamines play critical roles for cells and are involved in protein and DNA manufacture. The research project staff is developing a combination therapy involving a polyamine biosynthesis inhibitor difluoromethylornithine (DFMO) and a polyamine transport inhibitor (PTI). This full court press on polyamine metabolism starves these tumors of the growth factors they need to grow. More importantly,

this therapy has a profound effect on the immune system and stimulates immune cells to enter and kill the tumor cells. The progress to date includes the generation and validation of new PTI compounds which target specific modes of polyamine import, a world first. Using a mouse model of pancreatic cancer (e.g., orthotopic PanO2 cells), the team has shown that mice treated chronically with this combination therapy (DFMO plus PTI) have a doubling of survival compared to untreated mice. The team has also characterized a new polyamine transport protein (*ATPase 13A3*) which responds to polyamine stimuli by moving from the cell's nucleolus to the plasma membrane to take up polyamines from outside the cell. This fundamental understanding of the proteins used by cells to harvest polyamines from their environment will allow the team to design even more powerful interventions in the future.

Patients often die from metastatic disease, where the tumor cells have escaped the primary tumor and go on to inhabit a new tissue. Using circulating tumor cells (CTC) isolated from patients with pancreatic cancer at Advent Health Orlando, the research team has discovered that myeloid fibroblast (MFB) cells help the CTC cluster and migrate to other organs. Importantly, the team has also shown that the clustering ability of the CTC is altered in the presence of DFMO plus PTI therapy. Since clustering Is important to metastasis (tumor spread), disrupting this property may contribute the observed increased survival in the mouse models tested. In sum, the DFMO plus PTI therapy Increases overall survival by depleting polyamines in the tumor's microenvironment, which disrupts tumor cell cluster formation and allows immune cells to enter and kill the tumor. The impact on Floridians includes new hope that this promising therapy will advance to clinical trials to help patients with this devastating disease. The return on investment comes in the form of a newly filed U.S. patent covering this new technology and the ongoing creation of a technology package to attract big pharm to license this technology from the University of Central Florida (UCF) and its partners.

Follow-on Funding: Pancreatic Cancer Action Network; *Targeting Polyamine Metabolism in Pancreatic Cancers*; Otto Phanstiel; $500,000 (pending).

Collaborations: The primary research center for this grant is the University of Central Florida College of Medicine, Department of Medical Education. Students from the Biomedical Sciences PhD program are involved including graduate students: Aiste Dobrovolskaite and Sai Preethi Nakkina. Two post-doctoral researchers are also working on the project: Drs. Vandana Sekhar and Mukund Tantak.

Journals: None at the time of reporting.

Patents: Provisional U.S. patent application *(Non-Polyamine Based Polyamine Transport Inhibitors and Their Use in the Treatment of Human Cancers*, UCF docket# 10669-260 USO) was submitted in August 2018 to cover the new PTI architectures. University of Central Florida (UCF) filed the Invention.

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

Principal Investigator: Barry Hudson, PhD

Organization: University of Miami

Grant Progress Report: In the current funding period, the research team has made major progress with testing RAGE (*receptor for advanced glycation end* products) inhibitors in breast cancer cell models and in animal models of breast cancer. In cell assays, researchers have optimized assays for RAGE inhibitors on cell signaling pathways. They have shown different effects of RAGE inhibitors on tumor cell invasion and viability. Further, the research team has made new discoveries with respect to the administration of RAGE inhibitors in animal models and their impact on tumor cell growth and progression.

Follow-on Funding: Florida Department of Health; *Targeting of RAGE in Breast Cancer Progression*

and Metastasis; Barry Hudson, PhD; $815,000.

Collaborations: The research team is working with colleagues at Baylor University in Waco, Texas, to perform proteomic analysis on the effects of RAGE signaling

Journals: None at the time of reporting.

Patents: Researchers recently were granted a patent on their work using RAGE inhibitors and breast cancer. The title is as follows: “Method for treating breast cancer and chronic disease.” (Patent number: US 62/583,90)

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

Principal Investigator: Ashok Saluja, PhD

Organization: University of Miami

Grant Progress Report: Researchers hypothesize that exposure to gut microbiome induces immune suppression in the liver, which could lead to creation of a permissive environment for colon cancer metastases. The goals of this research project are as follows: 1) To elucidate the role of gut microbiome in modulating liver metastases; 2) To evaluate the role of immune modulation in gut microbiome induced promotion of liver metastases; and 3) To elucidate the role of toll-like receptors in gut microbiome induced enhancement of liver metastases.

So far, the research team has shown that gut microbiome promotes growth of tumors and liver metastases in various models of cancer, such as pancreatic cancer, colon cancer and melanoma.
The role of gut microbiome was further confirmed using germ-free mice (lacking gut microbiome). Using germ-free mice, researchers evaluated the effect of a gut microbiome transplant. For this, germ-free mice were gavaged with feces of specific-pathogen free (SPF) wild-type mice to generate conventionalized mice. The team observed that the conventionalized mice had bigger tumors compared to germ-free mice, another indication that gut microbiome has a role in tumor growth.

In order to make the findings therapeutically viable, researchers investigated whether individual antibiotics can produce the same effect as a combination of various antibiotics. The results suggest that individual antibiotics are as effective as a combination of multiple antibiotics.

Researchers are continuing to investigate the role of toll-like receptors TLR (TLR2 and TLR4 in colon cancer and metastasis). Their studies show that gut microbiomes possibly mediate their anti-tumor effect by interacting with TLR2 and TLR4 receptors. Studies were conducted using TLR2 and TLR4 agonists in colon cancer mice models (MC38 cell line).
To understand the mechanism by which the gut microbiome interacts with the immune system in improving tumor outcomes, researchers investigated the role of certain cytokines, such as Interleukin (IL)-17 and Interleukin (IL)-23, which are known to play a role in tumor immunity. Present studies show that gut microbiome inhibition (by using antibiotic cocktail treatment) leads to decreased IL-23 production, which in turn leads to decreased IL-17 production. This leads to a reduction in tumor sizes in pancreatic cancer mouse models. At present, experiments are underway to identify whether this scenario is true in colon cancer 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.

8. 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 in Florida using a technique called mass imaging cytometry (MIC). Historically, there have been various staining methods that allow pathology labs to identify various characteristics of tumors from a patient biopsy. However, 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 one to four 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, as 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.

The equipment was installed in August 2018. Four training sessions for staff (10 days total) occurred in September and November 2018, and twice in March 2019. Two research symposia were held to promote use of the equipment (October 2018 and June 2019), which were well attended (95 attendees total). Three grants were submitted during this period, including two prestigious National Institutes of Health (NIH) R01 grants and one Florida Academic Cancer Center Alliance (FACCA) grant. The FACCA grant was not awarded, but the NIH grants are still pending review.

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 up to $10,000 each to perform pilot studies using MIC. Thirteen innovative proposals were received, and five of them will be funded by the end of July 2019. Furthermore, SCCC is establishing a reagent bank to reduce the cost of reagents for researchers.

The MIC facility was viewed very favorably during review of the SCCC application to become a National Cancer Institute (NCI)-designated cancer center, which was recently approved. Researchers at SCCC are beginning to do pilot studies using MIC, and discussions are ongoing with researchers at H. Lee Moffitt Cancer Center and Research Institute in Tampa to utilize MIC as well. At the recent statewide scientific FACCA retreat (June 2019), the MIC facility was presented, and there was great interest in leveraging it for all cancer research in the state. It is expected that there will be much more scientific impact to Florida in year two of this grant as the facility becomes more established and pilot studies generate additional grant funding.

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 #8BC10: Multifunctional Nanoparticle for Targeted Combination Therapy of Prostate Cancer

Principal Investigator: Shanta Dhar, PhD

Organization: University of Miami

Grant Progress Report: Over the last few months, the researchers have started building their xenograft model to study the distribution and therapeutic potential of the nanoparticle platform. Animals have been inoculated with tumors, and the team is now waiting for the tumor to form to begin the study. Researchers also studied bone metastasis inhibition during this period of the grant and set out to build a robust method to quantify pamidronate (PAM), which is the bone metastasis inhibitor in their nanoparticle, with highest accuracy. First, they employed quantification using concentration-dependent quenching of fluorescence of an Al3+ Morin complex by the bisphosphonate pamidronate. A 1 *millimolar* (mM) solution of morin was prepared in 4:1 ethanol/water and aluminum nitrate was prepared in Nanopure water. The two solutions were mixed and diluted with water to a final concentration of 2 *micrometers* (µM) Al3+ and 10 µM morin.

The potential hydrogen (pH) of this solution was adjusted to 4.5 with an acetate buffer and was stored overnight at room temperature to allow the morin-Al3+ fluorescent complex to form. The following day, 150 microliters (µL) of this solution and 50 µL of the pamidronate standard solution were mixed in a 96 well plate, and the fluorescence was read immediately in kinetic mode with a plate reader, at 410/495 nanometers (nm)(ex/em.)

With this method, the research team observed non-specific binding to the polymer, poly(lactic-co-glycolic acid) (PLGA), which was used to synthesize PLGA-PAM. Additionally, generated standard curves were not linear enough to be used for accurate calculation of pamidronate conjugated to PLGA. The non-linear standard curves made this method ineffective at quantifying pamidronate. It is possible that the polyester bonds in PLGA are binding to the complex resulting in an inaccurate result.

Next, researchers used quantification by bisphosphonate complex formation with copper (II) sulfate. Pamidronate stock solution was made in water. Standard solutions were prepared in a range of concentrations by adding acetate buffer and 2 *milliliters* (ml) of 2.5 millimolar (mM) copper (II) sulfate solution and completing the dilution with Nanopure water. The absorbance values of the standards were measured at 264 nm against a reagent blank. The values of absorbance were then plotted against the concentration to generate a linear standard curve. In this process, the researchers discovered that the PLGA-PAM molecule created interference in the complex formation with copper (II) sulfate while pamidronate alone produced an accurate reading. The quantification of PLGA-PAM is not possible through this method.

Thus, the research team decided to utilize a hydrophobic prodrug of pamidronate instead of PLGA-PAM. The initial goal with PLGA-PAM was to make a hydrophobic analogue of pamidronate to increase encapsulation in nanoparticles. Keeping this goal, the researchers decided to create a different pamidronate prodrug, Lauric-Pamidronate, which will also make the pamidronate hydrophobic while alleviating the issues caused by PLGA in interfering with the complex formation. With this new prodrug, the researchers were able to preliminarily quantify pamidronate using a linear standard curve generated with pamidronate.

Pamidronate disodium hydrate (0:05 g, 0.179 millimoles [mMol] was dissolved in 10% acetic acid for 30 minutes. The solvent was then evaporated using a rotary evaporator. Lauric Acid (34 mg, 0.179 mMol) was activated by dissolving the acid in 3 milliliters of dry dimethylformamide (DMF). Then (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide [EDC] hydrochloride (35.8 mg, 0.187 mMol) and N-hydroxysuccinimide (NHS) (21.5 mg, 0.187 mMol) were added to the solution. The solution was stirred at room temperature for six hours. The previously dried pamidronate was dissolved in 1 milliliter of water

and added to the reaction mixture. The reaction was allowed to continue for 36 hours. The solvent was partially removed under vacuum and the remaining solution was dissolved in ethyl acetate. The resulting precipitate was removed, and the ethyl acetate was concentrated. This was then reprecipitated with hexanes twice, and then dried under high vacuum.

Prostate cancer is known to metastasize to the bone, where it induces the generation of osteoclasts, leading to increased bone resorption. This leaves the bone vulnerable to tumor growth. Tartrate resistant Acid Phosphatase (TRAP) is a marker present in osteoclast-like cells. Macrophage colony-stimulating factor (M-CSF) is a co-stimulator of osteoclastogenesis, which enhances the prostate cancer cell induced osteoclastogenesis, and OPG is a known inhibitor.

Lymph *node carcinoma of the prostate* (LNCaP) cells were plated at 10,000 cells/well in six well plates and grown for 24 hours. Freshly harvested murine bone marrow cells were collected and then added to the LNCaP cells at 10,000 cells/well and co-cultured for 24 hours. One *ng/ml of osteoprotegerin* (OPG) and 1 ng/ml macrophage colony-stimulating factor (M-CSF) were added to the same well, and M-CSF was added to the other wells for 24 hours. Cells were then treated with non-transduced (*NT)/T cells*-poly lactic-co-glycolic acid (PLGA)-Pam--nanoparticles (NPs) and NT/T-Tripeutic-NPs with concentration of 1 µM with respect to Platin-L. The NT/T-PLGA-Pam-NPs concentration was kept at 60 µg/mL with respect to polymer. The media were removed, cells were washed with phosphate buffered saline (PBS) (1X, two times) and cell lysates were collected using radioimmunoprecipitation assay (RIPA) buffer. The protein fractions were collected by centrifugation at 10,000 rpm for 20 minutes. The protein amounts in the cells were quantified using bicinchoninic acid (BCA) assay. Thirty µg of protein was used in each well along with 1X *Laemmli* sample buffer and the gel was run at 100 millivolts (mV) for 2 hours. The resolved proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane at 50 mV, 4 degrees Celsius for two hours. The membrane was blocked for one hour in blocking buffer made with 5% skim milk powder in tris-buffered saline tween (TBST, tris=2.42 grams and NaCl (sodium chloride)=8.0 gin one liter of deionized (DI) water at pH of 7.6; *Tween 20 (1:1000 dilution*). The membrane was kept at 4 degrees Celsius overnight for primary antibody (tartrate-resistant acid phosphatase [TRAP] and tubulin incubation. The next day, the membrane was washed five times with TBST buffer and then incubated with respective secondary antibodies at room temperature for one hour. The membrane was again washed five times with TBST buffer before developing with EGL, and the images were taken using the BioRad ChemiDoc™ imaging system. The bands in the membrane were quantified by Image J software. TRAP is a marker present in multinucleated osteoclast-like cells. M-CSF is a co-stimulator of osteoclastogenesis that enhances the prostate cancer cell-induced osteoclastogenesis. OPG is an inhibitor of prostate cancer-induced osteoclastogenesis.

To study TRAP levels by immunofluorescence, LNCaP cells were plated at 10,000 cells/well in 12 well-plates on cover slips and grown for 24 hours. Freshly harvested murine bone marrow cells were collected and then added to the LNCaP cells at 10,000 cells/well and co-cultured for 24 hours. Media were replenished with fresh media. One ng/mL OPG was added to one well and 1 ng/mL M-CSF was added to the other wells for 24 hours. Once every two days, media were replenished and fresh OPG and M-CSF were added. Three cycles of this treatment were done. Cells were then treated with *NT/T*-PLGA-Pam-NPs and NT/T-Tripeutic-NPs with a concentration of 1 µM with respect to Platin-L. The NT-T-PLGA-Pam-NPs concentration was kept at 100 µg/ml with respect to polymer. The cells were washed with 1X PBS three times and fixed with 4% paraformaldehyde for one hour at 37 degrees centigrade. After performing three washings, cells were permeabilized using 0.1% Triton-X100 for 10 minutes at 37 degrees Celsius. The cells were washed with 1X PBS three times and blocked with 1% goat serum in 1X PBS for 12 hours. Cells were treated with the respective primary antibody (TRAP antibody) in 5% goat serum containing 1X PBS for 12 hours at 4 degrees Celsius in humidified chamber. After washing the cells for three more times with 1% goat serum containing 1X PBS, the respective secondary antibody (Alexa 488 conjugated anti-mouse antibody) solution in 1% goat serum containing 1X PBS was added at room temperature. Cells were stained with 4',6-diamidino-2-phenylindole (DAPI) for five minutes. Cells were finally washed five more times with 1% goat serum containing 1X PBS. The membrane was gently

removed and kept on glass slides and covered with coverslips using mounting solution (n-propyl gallate, tris and glycerol in Nanopure water, pH = 8.0). Confocal images were recorded using an Olympus FluoView FV3000 confocal microscope using 405/460 nm for DAPI and 488/510 nm for Alexa 488. The data showed that M-CS-treated cells show an increase in TRAP positive cells compared to cells treated with both M-CSF and OPG. Additionally, T-Tripeutic-NPs showed a decrease in TRAP positive cells, indicating that they are inhibiting osteoclastogenesis. Other NP treated groups show a decreased amount of TRAP positive cells compared to the M-CSF treated control.

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 the reporting.

10. Grant #8BC12: Tissue Microenvironment and Tumorigenesis Hotspots

Principal Investigator: Wu-Min Deng, PhD

Organization: Florida State University

Abstract of Proposed Research: In epithelial tissues, cells communicate with their neighbors and receive information from the surrounding environment through signaling networks, adhesion molecules and junctional molecules in order to form complex organs and to maintain their integrity and morphology. This robust self-organizing system, however, is progressively disrupted during tumor development. In tumorigenesis, transformed mutant cells evolve into a malignant neoplasm through a multistep process, whereby the transformed cells acquire traits that enable them to become tumorigenic and ultimately malignant. Although many genes have been identified as involved in different steps of cancer cell progression, little is known about the beginning of tumorigenesis, wherein mutant cells deviate from the robustly organized microenvironment to evolve into aggressive tumors.

In the researchers' analysis of conserved neoplastic tumor-suppressor genes (nTSGs) using the Drosophila wing imaginal disc model system, the researchers found specific regions in which tumors always originate. Within this system, specifically, they looked at the epithelial folds of the wing hinge region. In these "tumor hotspots," pro-tumor nTSG mutant cells delaminate from the apical side of the epithelium and start tumorigenic overgrowth by exploiting endogenous Janus kinase/signal transducers and activators of transcription (*JAK*/*STAT*) inflammatory signaling activity. In contrast, these pro-tumor cells in tumor coldspots are normally extruded from the basal side of the epithelial layer and undergo apoptosis. The research shows tumor hotspots display a network of specific and robust basal structures, including a web of intertwining filopodia and enriched basal microtubule cytoskeleton. The researchers hypothesize that the cytoarchitectural structures in "tumor hotspots" force pro-tumor cells to delaminate from the apical side and enter the lumen, where JAK/STAT activity is high, allowing tumorous growth in a "niche-like" environment.

The research team plans to test the hypothesis and, thereby, to accomplish the objectives of the application by pursuing the following two specific aims: 1) determine how the direction of nTSG-loss-of-function (LOF)-cell delamination is regulated and its relationship to dysregulated cell proliferation; 2) determine how JAK-STAT signaling is involved in tumor hotspot formation; and 3) determine the role of cell competition in tumor hotspot and coldspot differentiation. The expected outcomes from these aims will advance understanding of mechanisms underlying the initial stages of tumorigenesis. Given the conservation of the epithelial cytoarchitecture, carcinogenesis may be generally initiated from tumor hotspots by a similar mechanism. An example of the tumor hotspot in mammals is the "transition zone,"

where two different types of epithelial tissue meet. This results in the appearance of a distinct abrupt transition, which can be found in numerous locations within various tissues, such as the region between the periphery and central zones in the prostate, and the intestinal mucosa (colorectal zone) changes to anal mucosa (anoderm) in the anal canal. Understanding these regulatory mechanisms will therefore provide new insights into how tissue-intrinsic microenvironment determines whether tumors can be induced after cells acquiring cancer-promoting mutations. The proposed studies will ultimately propel the development of new therapeutic avenues for preventing cancer development or managing cancer at an early stage.

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.

APPENDIX C

FISCAL YEAR 2018-2019 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
7BC01	University of Miami	Capobianco, Anthony	$ 1,471,318	$ 1,021,748.25	$ 449,569.75	3/17/2017	2/29/2020	Yes	No	No
7BC02	University of Florida	Judge, Andrew	$ 1,226,836	$ 851,968.75	$ 374,867.25	3/22/2017	2/29/2020	No	No	No
7BC03	University of Miami	Thomas, Emmanuel	$ 1,866,436	$ 777,221.25	$ 1,089,214.75	3/17/2017	2/28/2022	No	Yes	Yes
7BC04	H. Lee Moffitt Cancer Center	Gwede, Clement K.	$ 828,125	$ 345,050.00	$ 483,075.00	3/15/2017	2/28/2022	No	No	Yes
7BC05	H. Lee Moffitt Cancer Center	Smalley, Keiran	$ 1,468,200	$ 978,800.00	$ 489,400.00	4/12/2017	2/29/2020	No	Pending*	No
7BC06	Florida Atlantic University	Wright, Amy E.	$ 622,683	$ 412,084.90	$ 210,598.10	3/27/2017	2/29/2020	No	No	No
7BC08	H. Lee Moffitt Cancer Center	Pilon-Thomas, Shari	$ 976,620	$ 678,200.00	$ 298,420.00	3/08/2017	2/29/2020	No	No	No

*Articles have been accepted for publication 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 Capobianco, PhD

Organization: University of Miami

Grant Progress Report: In order to more efficiently screen novel NACK (Notch transcriptional activation complex kinase) inhibitors, the research team developed the first NACK binding assay via FQCR (fast quantitative cysteine reactivity). This label-free binding experiment measures the melting temperature of the protein by detecting fluorescence of a cysteine dye. Researchers were able to extract a KD (dissociation constant) from the difference in the melting temperature of the protein with and without treatment with compound.

The research team estimated that its initial hit (compound Z271-0326, or iNACK) binds with a KD of 1.5 millimolar (mM) and improved analogue UM-88, a type II inhibitor, binds with a KD of 300 nanomolar (nM). Z271-0326 and the analogue compounds are validated and screened by cell-based assays in different cell lines: esophageal adenocarcinoma OE33 cell lines are used in colony formation assay, while triple negative breast cancer uses MDA MB 231 cell lines in the CellTiter-Glo assay. Z271-0326 and UM-88 significantly affect the viability of these cell lines: The IC50, calculated by colony formation assay, is around 3 micromolar (uM) for Z271-0326 and 1.5uM for UM-88. This reflects the team's data about the analogue binding to the protein.

Additionally, researchers have adapted an affinity pull-down assay to study the Adenosine triphosphate (ATP) significance in the NACK dimerization process. They demonstrated that an ATP molecule attached to an agarose bead will bind NACK. However, a NACK mutant that prohibits dimerization did not bind to the ATP bead, suggesting that dimerization is necessary for ATP binding. The NACK-iNACK binding hypothesis has been strengthened by the analogue UM-88. Researchers now believe that this type II inhibitor has the Boc carbamate group sticking toward the back of the C-alpha helix, with the pyrazolopyridine facing the hinge region. Current compound optimization has been focused toward exploring different scaffold modifications. The piperazine analogue has been synthesized, and the route is being optimized. Progress has been made toward the synthesis of the imidazolo and triazolo scaffold modifications.

Follow-on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: *Inhibitors of the Notch Transcriptional Activation Complex Kinase ("NACK") and Methods for Use of the Same* - U.S. Provisional Patent Application No. 62/626,870

2. Grant #7BC02: Initiating Mechanisms of Cancer Cachexia

Principal Investigator: Andrew R. Judge, PhD

Organization: University of Florida

Grant Progress Report: Cachexia is a devastating effect of many cancers that is characterized by the unintentional loss of body weight due to the loss of skeletal muscle. Cachexia negatively impacts physical function and functional independence and thus quality of life. In addition, cachexia impacts

tolerance to cancer therapies and is predictive of poor outcomes and decreased survival time. In general, cachexia is believed to be induced by factors released from tumors and/or host cells. Therefore, this research is focused on identifying such factors released from cancer cells and establishing their causality in cancer cachexia. In this regard, the research team has identified that human pancreatic tumor cells release high levels of the proteins C-X-C motif chemokine ligand 8 (CXCL8, or IL-8) and C-X-C motif chemokine ligand 1 (CXCL1). These proteins are both members of the CXC chemokine family and can signal through the same receptor—C-X-C chemokine receptor 2 (CXCR2). Interestingly, the CXCL8 gene is lacking in the muroid lineage, but mouse colon adenocarcinoma (C26) cells, Lewis lung carcinoma (LLC) cells, and pancreatic KrasG12D; Trp53R172H; Pdx1-Cre (KPC) cells, all of which induce cachexia in mice, each release CXCL1. Since there is currently no data on the role of CXCL8 or CXCL1 in the regulation of skeletal muscle mass, the research team is studying these proteins in this regard. Research staff have found that treatment of skeletal muscle cells, or mice, with recombinant human CXCL8 or CXCL1, or mouse CXCL1, can induce significant atrophy of limb and respiratory muscles. In muscle cells, this is associated with activation of extracellular-signal-regulated kinase (ERK) 1/2, signal transducer and activator of transcription 3 (STAT3), and SMAD3, which have each previously been implicated in cancer cachexia. However, using specific inhibitors of each, the team identified that only ERK1/2 signaling is required for CXCL8 and CXCL1-induced atrophy. Since the CXCR2 receptor is increased in the muscles of tumor-bearing hosts and is a viable target to inhibit both IL-8 and CXCL1 signaling, researchers have conducted experiments to inhibit this receptor.

Researchers generated skeletal muscle specific CXCR2 knockout mice ($CXCR2^{SkmKO}$) and implanted pancreatic cancer cells. However, muscle atrophy was comparable in $CXCR2^{SkmKO}$ mice and wild type mice, suggesting that CXCR2 expression on muscle cells is not required for cancer-induced muscle atrophy. An adeno-associated viral vector expressing a shRNA targeting CXCR2 was then injected into the skeletal muscle of mice that were subsequently implanted with human pancreatic tumor cells. This approach targets all cells within skeletal muscle. shRNA-CXCR2 injection inhibited the increase in CXCR2 levels and blocked the tumor-induced skeletal muscle wasting. To complement these genetic experiments, mice bearing pancreatic tumors with a CXCR2 inhibitor were treated and an attenuation of muscle atrophy compared to non-treated mice was found.

These findings suggest that signaling through CXCR2 is required for muscle wasting in response to human pancreatic tumors. Since CXCR2 inhibitors are being tested in phase 1 clinical trials, the results create an opportunity for such inhibitors to be tested as anti-cachexia drugs. Given that no anti-cachexia drugs are currently available, this represents an exciting opportunity.

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

Principal Investigator: Emmanuel Thomas MD, PhD, FAASLD

Organization: University of Miami

Grant Progress Report: The research team has collected clinical information and now has a comprehensive database for 2,080 patients with liver disease who are at increased risk of developing hepatocellular carcinoma (HCC). As described in Aim No. 1 of the grant, researchers have completed the

cross-sectional analysis that will be carried out in the 2,080 patients to identify novel clinical covariates that may drive liver disease progression. The 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. Future work will focus on Floridians with high and intermediate risk of developing HCC and try to generate a new risk calculator that incorporates FibroScan.

The study team has begun to develop new non-invasive prediction models for fibrosis and cirrhosis toward its efforts to develop a liver cancer risk calculator that utilizes race/ethnicity. 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, the study team believes these efforts will lay the foundation for future work. Using multivariable statistical modeling, researchers can accurately predict cirrhosis (Metavir F4 fibrosis stage) utilizing noninvasive clinical markers and are now mapping liver disease based on zip code-based data.

Importantly, researchers have submitted follow-on grants to the National Institutes of Health (NIH), the Department of Defense (DOD) and a recent application for a Bankhead Infrastructure grant that will take their work and expand it to the rest of Florida by leveraging the OneFlorida consortium based at the University of Florida. Additionally, since starting this Bankhead-Coley Grant, Dr. Thomas has been awarded a five-year, renewable grant from the National Institutes of Health (NIH) for $1.9 million. The NIH -funded study is focused on understanding inflammatory mechanisms 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 receiving a minority supplement to support a graduate student. In addition, Dr. Thomas has been awarded a new $300,000 grant from Gilead Sciences to screen for hepatitis C virus (HCV) and human immunodeficiency virus (HIV) in the University of Miami Division of Emergency Medicine. Furthermore, the research team recently established the Florida HCV-HCC/Liver Cancer Consortium with H. Lee Moffitt Cancer Center and Research Institute, the University of Florida, and Jacksonville Mayo Clinic through three meetings.

Follow-on Funding: None at the time of reporting.

Collaborations: Researchers recently formed the Florida HCV-HCC/Liver Cancer Consortium with Moffitt Cancer Center: Dr. Anna Giuliano, founding director of the *Center* for Infection Research in Cancer (CIRC); Dr. David Nelson, director of the UF Clinical and Translational Science Institute; Dr. Betsy Shenkman, chair of the Department of Health Outcomes and Biomedical Informatics and the co-director of the University of Florida Clinical and Translational Science Institute; and Dr. Tushar Patel, gastroenterologist (transplant hepatologist) Mayo Clinic Jacksonville. The consortium has met on four occasions: at Moffitt Center in Tampa in October 2017; in Miami on May 7, 2018; at the University of Florida in Gainesville on April 19, 2019; and in Orlando on August 16, 2019.

This project is currently providing training to four University of Miami graduate students: Alexandra Debose-Scarlet (third-year medical student, MD program); Jasmine Edwards (third-year graduate student, PhD program); Owen Willis (second-year graduate student, PhD program); and Alejandro Badilla (second-year graduate student, PhD program). Three University of Miami undergraduate students (David Barr, Danae Lally and Shree Patel) are also receiving training under this project.

Journals: DeBose-Scarlett A, Balise R, Kwon D, Vadaparampil S, Chen SX, Schiff ER, Ayala GP, Thomas EJ. Obstacles to successful treatment of hepatitis C in uninsured patients from a minority population. *Transl Med.* 2018 Jun 28;16(1):178. doi: 10.1186/s12967-018-1555-y. PMID: 29954391.
Kasting ML, Giuliano AR, Reich RR, Roetzheim RG, Duong LM, Thomas E, Nelson DR, Shenkman E, Vadaparampil ST. Hepatitis C virus screening trends: A 2016 update of the National Health Interview Survey. *Cancer Epidemiol*. 2019 Apr 3;60:112-120. doi: 10.1016/j.canep.2019.03.007. PMID: 30953971.
Thomas, E, Baumert TF. Hepatitis B virus-hepatocyte interactions and innate immune responses:

experimental models and molecular mechanisms. *Semin Liver Dis*. 2019;39(3):301-314. doi: 10.1055/s-0039-1685518. PMID: 31266064.
Hyun J, McMahon RS, Lang AL, Edwards JS, Badilla AD, Greene ME, Stone GW, Pallikkuth S, Stevenson M, Dykxhoorn DM, Kottilil S, Pahwa S, Thomas E. HIV and HCV augments inflammatory responses through increased TREM-1 expression and signaling in Kupffer and myeloid cells. *PLoS Pathog*. 2019 Jul 1;15(7). PMCID: PMC6625740.

Patents: None at the time of reporting.

4. Grant #7BC04: Community CARES: A Multilevel Intervention to Increase Colorectal Cancer Screening Adherence in Community Clinics

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

Organization: H. Lee Moffitt Cancer Center and Research Institute

Grant Progress Report: This project has two phases. Phase one (the preparatory phase) focuses on conducting activities that provide important initial processes to support the conduct of phase two activities (randomized clinical trial [RCT]). Phase one was completed in previous reporting periods, and phase two activities are ongoing. As such, this progress report centers on continuation activities for phase two as summarized below.

For phase two, RCT started at clinics on March 29, 2018. Below are the aims with their progress to date:

The first aim was to test whether C-CARES (Community Colorectal Cancer Awareness, Research, Education and Screening) Plus (education plus fecal immunochemical test (FIT) plus personalized components) compared with C-CARES (only education plus FIT) improves long-term FIT screening adherence among 328 individuals, 50-75 years of age, who are not up-to-date with colorectal cancer screening (CRC).

A total of 2,133 age-appropriate (50-75 years old) patients were recruited to participate. Of these, 753 were not interested in the study. A total of 1,380 patients were then evaluated for full eligibility. Of these, 1,108 were ineligible for a variety of reasons with the primary reason (74%; 816/1108) being up to date with their CRC screening. This left 272 patients who were eligible for the study. A total of 251 were successfully enrolled. (Of the 21 not enrolled, 11 are pending enrollment, and 10 were lost to enrollment). Also unadjusted FIT uptake (both arms combined) reflects 63% as of May 31, 2019, not accounting for follow-up time.

No intervention comparisons are planned until completion of accrual and interval follow-up. Overall, since the start of accrual, there have been a total of 30 abnormal FIT results, and 14 patients have completed colonoscopy screening. The remaining 16 patients are being tracked for colonoscopy completion either by the Moffitt Cancer Center team or navigated through the clinics' usual care practice. The cumulative accrual to date (51/328) represents 77% of projected accrual.

As such, based on the current pace of accrual, research staff anticipate completing accrual of 328 within this calendar year or sooner as study staff resources will be deployed to the clinic sites with the highest patient volumes and the highest accrual yield.

For the C-CARES Plus arm, a three-month coaching intervention has been implemented. A total of 36 participants were due for coaching. Of those, 21 received the coaching intervention, three are pending, and 12 could not be reached in the prescribed interval per protocol. The 12-month follow-up and booster

education interactions began in April 2019. In total, 19 participants were contacted, 15 completed follow-ups, and four are pending. For participants in the C-CARES arm, 23 were mailed a generic CRC message via postcard per protocol. No other planned interactions or assessments between participants and study coordinators occur until final follow-up (27 months).

As far as impact to Floridians, the produced educational DVD and photo novella 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.

Follow-on Funding: Florida Blue; *Moffitt Cancer Center Community CARES*; Clement K. Gwede, PhD**;** $250,000

Collaborations: Two Moffitt-based post-doctoral fellows were involved in the project as part of their training under the p*ostdoctoral* training *program* in *behavioral oncology* (R25T), under the mentorships of Drs. Gwede and Cathy Meade. Both trainees have since completed their tenure at Moffitt Cancer Center and moved to other institutions.

Journals: None at the time of reporting.

Patents: None at the time of reporting

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

Principal Investigator: Keiran Smalley, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Grant Progress Report: The purpose of this work is to develop new therapeutic approaches for uveal melanoma, the most common and devastating form of eye cancer. Currently, there are no effective treatments for uveal melanoma, and most patients with advanced disease will die from liver metastases. The most promising therapy to be evaluated in cases of advanced uveal melanoma is a class of drugs called MEK (mitogen-activated protein kinase kinase) inhibitors. Although these drugs are initially effective, patients typically only respond for a few months before relapsing. The central goal of this ongoing research is to develop combination drug strategies that increase the effectiveness of MEK inhibitors in uveal melanoma.

In preliminary studies, the research team identified a class of enzymes called histone deacetylases (HDACs) that were found to be critical in the development of resistance to MEK inhibitors. The team includes Dr Keiran Smalley (Moffitt Cancer Center), an expert on melanoma biology and therapy; Dr. Jonathan Licht (University of Florida Cancer Center), an authority on epigenetics and clustered regularly interspaced short palindromic repeats (CRISPR) screening; and Dr. J. William Harbour (University of Miami,) an ophthalmic surgeon and eye cancer expert.

Three aims were proposed. The goal of the first aim was to define the epigenetic landscape of uveal melanoma using ATAC-Seq and RNA-seq and explore how this landscape was regulated by three genes: GNAQ, BAP1 and HDAC8. The second aim will focus on how the three genes (e.g., GNAQ, BAP1, and HDAC8) regulate signaling networks in uveal melanoma cells and how these networks become rewired following treatment with MEK inhibitors. The goal of the third aim is to test whether the combination of a MEK and an HDAC inhibitor could be developed as a novel therapy for uveal melanoma.

In the second year of the grant, the research team has made significant progress towards achieving

these aims. The group has completed and published its first paper in the journal Clinical Cancer Research. In this manuscript, Dr Smalley's lab demonstrated that MEK inhibition leads to the activation of survival pathways in uveal melanoma cells. Through use of a drug screen and proteomic methods, novel drugs, including the FDA-approved HDAC inhibitor panobinostat, were demonstrated to limit therapeutic escape following treatment with a MEK inhibitor. The efficacy of the MEK-HDAC inhibitor combination was confirmed in cell culture models and a clinically relevant mouse model of uveal melanoma liver metastasis. These findings provide the groundwork to develop this drug combination clinically. Progress has also been made toward Aim No. 1, with much of the work on how mutant GNAQ and BAP1 regulate the epigenome and signaling networks in uveal melanoma cells now being complete. The data are being integrated to determine new druggable targets in uveal melanoma cells. As a final achievement, Dr. Licht's lab has completed the first screen (a CRISPR screen) to identify the genes required for uveal melanoma growth and survival. His lab has identified a list of important targets that the group will validate in the third year of the grant.

Follow-on Funding: Melanoma Research Alliance (Team Science Award); *Developing Drivers of Melanoma Metastasis***;** Keiran Smalley, PhD; $1,000,000 (Pending).

Collaborations: This project is a collaboration between Moffitt Cancer Center, the University of Florida, and the University of Miami. The groups continue to hold monthly Webex meetings to discuss data and progress. The group meets in person three-to-four times per year, more recently at the Miami Epigenetics and Cancer Symposium in November 2018, the American Association for Cancer Research (AACR) annual meeting in Atlanta in April, and the Florida Academic Cancer Center Alliance (*FACCA*) Research *Retreat* IV in Miami in June 2019.

Journals: Flores, F, Emmons, M, Durante, M, Saha, B, Fang, B, Koomen, JK, Maria-Engler, SS, Licht, JD, Harbour, JW, Smalley, KSM. HDAC inhibition enhances the in vivo efficacy of MEK inhibitor therapy in uveal melanoma. *Clin Cancer Res.* Published online: June 21, 2019. doi: 10.1158/1078-0432.CCR-18-3382.

Patents: None at the time of reporting.

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

Principal Investigator: Amy E. Wright PhD, MS, BS

Organization: Florida Atlantic University

Grant Progress Report: Survivin is a protein that has been identified as an important target for intervention in several cancers, including breast, colon and lung. Its presence in cancer cells correlates to poor prognosis and plays a role in the aggressiveness of these diseases. Survivin has several functions in cancer, including preventing cancer cell death (apoptosis), driving cell division (mitosis), providing nutrients to the tumor (angiogenesis) and contributing to resistance to existing medicines. It is not found in normal non-dividing adult tissues; therefore, drugs targeting survivin may provide selectivity toward cancer cells. Natural products are specialized chemical compounds produced by plants, animals, and microbes that provide advantages to the producing organism. They have been important as medicines to treat many diseases. Approximately 50% of the drugs currently used to treat cancer are based upon compounds found in nature. The Harbor Branch Oceanographic Institute (HBOI) of Florida Atlantic University has an extensive library of marine organisms which are a rich source of natural products. The goal of this project is to test materials from the HBOI natural products library for their ability to reduce the

levels of survivin in cancer cells, thus eliminating survivin's cancer protective properties. Discovery of such compounds will advance this field both in the understanding of basic biology of survivin and in eventual clinical practice.

The project has four specific Aims: 1) Develop high content imaging (HCI) assays to detect compounds that reduce levels of survivin in A549 (lung) or DLD-1 (colon) cancer cells; 2) Screen up to 3,000 materials from the HBOI library in each assay; 3) Identify the active compounds using bioassay-guided fractionation and advanced spectroscopic methods; and 4) Characterize the effects of the active compounds on apoptosis, signaling, metastasis, drug resistance, and possible molecular targets.

To date, assay development has been completed and 2,985 fractions have been tested in the DLD-1 and A549 HCI assays. One hundred seventy fractions met the criteria of reducing survivin levels by more than 50% while having low cytotoxicity. Many of the fractions are substantially more potent than known survivin inhibitors. Chemical investigation to purify and identify the structures of the active natural products in the most potent fractions has led to the identification of 14 active compounds of which seven are novel chemical structures. Purification and structure elucidation of additional compounds are underway. In addition to the compounds identified from chemical fractionation, eight compounds from the pure compound library reduced the levels of survivin in the DLD-1 colon cancer HCI assay. These results have been confirmed for seven of the compounds using Western analysis (protein immunoblot). Excitingly, the structures of the compounds are highly varied and do not resemble compounds known to reduce levels of survivin.

In the coming year research staff will further investigate the effects of the compounds in cancer cells and how they reduce levels of survivin. This will allow the team to move the compounds toward clinical development to help Floridians suffering from cancer.

Follow-on Funding: None at the time of reporting.

Collaborations: Kristie Tandbeg, a PhD candidate in the Integrative Biology program at Florida Atlantic University, Charles E. Schmidt College of Science, is performing research under this project. Emily Simon, an undergraduate who participated in the HBOI Summer Internship Program, also assisted with this project. Ms. Simon is from Duquesne University, Department of Biology, in Pittsburgh.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

7. 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: H. Lee Moffitt Cancer Center and Research Institute

Grant Progress Report: Melanoma is a leading cause of cancer mortality in the United States. Patients with melanoma and other cancers have immune cells (T cells) that can recognize and kill tumor cells. These T cells are ineffective due to suppressive factors in the cancer patient that allow tumors to "escape" recognition by T cells. These factors include myeloid derived suppressor cells (MDSC) that actively shut off T cell responses. One strategy to improve immune responses against tumors is adoptive cell therapy (ACT) using tumor-specific T cells. In this strategy, T cells are isolated from patient tumors and expanded in the laboratory to high numbers. This process allows the T cells to become reactivated and capable of mediating tumor killing. The expanded T cells are transferred back to the patient. ACT

with tumor-specific T cells has emerged as one of the most powerful therapies resulting in a 50% response rate in patients with unresectable metastatic melanoma. For this therapy to be effective, the patient must be treated with drugs that induce lymphopenia (depletion of circulating white blood cells). Induction of lymphopenia is important as it creates extra space for the transferred T cells to survive and proliferate. Lymphopenia is a temporary state and white blood cells will begin to repopulate the blood within a week after T cell transfer. Preliminary results show that MDSC recover quickly after the induction of lymphopenia and are even more suppressive than prior to induction of lymphopenia. This rapid repopulation of highly suppressive MDSC may decrease the effectiveness of ACT by shutting off T cells and preventing complete tumor regressions. MDSC are split into two different subsets and include monocytic MDSCs (M-MDSCs) and polymorphonuclear MDSCs (PMN-MDSCs). Expansion of both subsets is measured after induction of lymphopenia in both murine tumor models and melanoma patients treated on adoptive T cell therapy protocols. Potent suppression of T cell responses in both mice and humans treated with chemotherapy to induce lymphopenia has been measured in initial experiments.

In this study, researchers have evaluated the role of myeloid-mediated immunosuppression induced by lymphodepletion and its impact on ACT. They demonstrated that MDSCs rapidly accumulate within one week after completion of lymphodepleting regimens in mouse models and cancer patients receiving ACT with autologous T cells. Researchers determined that hematopoietic progenitors mobilized by lymphodepleting chemotherapy differentiate into MDSCs. Interleukin-6 (IL-6) was determined to be a key factor involved in the differentiation, survival, and immunosuppressive capacity of MDSCs induced by lymphodepletion. Furthermore, researchers show that lymphodepletion-induced MDSCs in patients receiving tumor infiltrating lymphocyte (TIL) therapy have direct impact on T cell function and patient survival. To date, researchers have results that support that reactive myelopoiesis in response to lymphodepleting chemotherapy initiates the rapid expansion of MDSCs that negatively impacts the efficacy of ACT.

Follow-on Funding: None at the time of reporting.

Collaborations: Pasquale Patrick Innamarato, a graduate student in the cancer biology PhD program at the University of South Florida, is performing research under this project.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

APPENDIX D

FISCAL YEAR 2018-2019 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	Tran, David D.	$ 1,784,753.25	$ 1,069,869.44	$ 714,883.81	3/04/2016	3/31/2021	Yes	No	Yes
6BC06	University of Miami	Antoni, Michael	$ 1,784,945.19	$ 1,076,919.19	$ 708,026.00	4/15/2016	3/31/2021	No	Yes	No
6BC09	University of Florida	O'Dell, Walter	$ 1,445,736.61	$ 858,529.94	$ 587,206.67	3/19/2016	3/31/2021	Yes	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 research team successfully completed Aim No. 1A, which detailed the preclinical study examining the combination of the p38 inhibitor PH-797804 and the cytotoxic agent 5-*fluorouracil* (FU). The final survival data at the 200-day mark was presented. Consistent with the hypothesis, pretreatment with the human p38alpha inhibitor PH-797804 reduced total bone marrow disseminated tumor cells (DTC) in triple negative breast cancer (TNBC), significantly increased overall survival, and resulted in a 40% cure, compared to 5-FU alone. However, since the research staff was unable to secure a clinical supply of a p38 inhibitor, as these agents are no longer in clinical development by pharmaceutical companies, the clinical trial as proposed and planned could not begin. Instead, researchers refocused their efforts on developing computational technologies that were part of the original Specific Aim No. 3. The researchers have now secured additional funding from the National Institutes of Health/National Cancer Institute (NIH/NCI) using the preliminary data generated in the first two years of this grant to proceed with testing their original concept that DTCs can be reawakened and thus eliminated by conventional chemotherapy. In this new study, an Interleukin 6 (IL-6) receptor inhibitor, which is FDA-approved for another indication and commercially available, will be used instead. The study is planned to start by the end of this year.

The research team has made significant progress toward, and in many instances far exceeded, many of the initial goals of Specific Aim No. 3. Specific Aim No. 3 is targeted at developing computational methods to analyze paired samples of primary breast tumors, DTCs and metastatic foci obtained from the planned clinical study. Through the development of these bioinformatics algorithms, research staff will create a major focus on computational biology for the University of Florida (UF) and the state of Florida that can be leveraged not only for the original proposal in TNBC but also for many other cancers and for biomedical research in general. In 2018, research staff completed optimization of some of the innovative computational algorithms developed in-house and are poised to begin to apply it to address important questions in cancer biology and therapeutic development.

On Feb. 8, 2019, researchers conducted an initial limited rollout at UF of a beta web-based version of NETZEN, their innovative artificial intelligence technology developed using the Bankhead-Coley grant. It was well received. Research staff has established collaboration with six independent groups at UF, completed all analysis for this first round of collaboration and is awaiting validation of these predicted targets experimentally. Improvements to NETZEN are continuously performed during this rollout based on feedback received from collaborators. The goal is to complete beta testing by the end of the year. This represents great progress on the experimental plan of functionalizing NETZEN, which will be a tremendous resource for investigators of all different areas of biomedical research at UF, in the state of Florida, and nationally as well.

Follow-on Funding: NIH/NCI; *Novel Methods of Chemo-Sensitizing Low-Proliferative Disseminated Tumor Cells in Triple Negative Breast Cancer;* David Tran, PhD; $3,410,247 (Pending). (This grant is based on preliminary data obtained during the first 2 years of 6BC04 and will test using IL-6/R inhibitor instead of p38 inhibitor as proposed in the original 6BC04 proposal.)

NIH/NCI; *Targeting Glioblastoma Stem-Like Cells With Custom-Designed Viral Vectors;* Kenneth Warrington, David Tran, PhD, and Sergei Zolotukhin, PhD; $2,300,000.

NIH/NCI; *Pglyrp3 Cooperates with Snail1 to Mediate Anti-Tumor Immune Response in Breast Cancer;* Matthew Sebastian; $180,010. (This grant is from a MD/PhD student working on breast cancer and the role of Snail1's pathway in regulating breast cancer metastasis and dormancy. Pglyrp3 was predicted as

an important downstream factor of Snail1 by GeneRep-nSCORE. The results from this work will be combined with the p38 and IL-6 data for our upcoming planned major publication.)

Collaborations: The following collaborators started various projects with the research staff based on the application of NETZEN to their respective work: Daohong Zhou, MD, College of Pharmacy; Yi Qiu, PhD, College of Medicine, Department of Anatomy and Cell Biology; Zhijian Qian, PhD, College of Medicine, Department of Medicine; Eric Vitriol, PhD, College of Medicine, Department of Anatomy and Cell Biology; Edgardo Rodriguez, PhD, College of Medicine, Department of Neuroscience, Lacerta Therapeutics; and Keith March, MD, PhD, director of the UF Center for Regenerative Medicine, College of Medicine, Department of Medicine.

The following collaborators are working with the research staff to advance the IL-6/R inhibitor trial to funded by the NCI: Karen Daily, MD, College of Medicine, Department of Medicine, Breast Oncology Program; and Hugh Fan, PhD, College of Engineering.

The research staff has also teamed up with Dr. Jose Trevino to take advantage of his large human tumor patient-derived xenograft (PDX) bank including more than 25 breast cancer PDX models. This PDX bank will be invaluable for validation experiment and therapeutic screening for the breast cancer master regulators identified by their computational platform.

Journals: Morley CD, Ellison ST, Bhattacharjee T, O'Bryan CS, Zhang Y, Smith KF, Kabb CP, Sebastian M, Moore GL, Schulze KD, Niemi S, Sawyer WG, Tran DD, Mitchell DA, Sumerlin BS, Flores CT, Angelini TE. Quantitative characterization of 3D bioprinted structural elements under cell generated forces. *Nat Commun*. 2019 Jul 10;10(1):3029. doi: 10.1038/s41467-019-10919-1. PMID: 31292444. PMCID: PMC6620298.

Galanis E, Anderson SK, Twohy EL, Carrero XW, Dixon JG, Tran DD, Jeyapalan SA, Anderson DM, Kaufmann TJ, Feathers RW, Giannini C, Buckner JC, Anastasiadis PZ, Schiff D. A phase 1 and randomized, placebo-controlled phase 2 trial of bevacizumab plus dasatinib in patients with recurrent glioblastoma: Alliance/North Central Cancer Treatment Group N0872. *Cancer*. 2019 Jul 10. doi: 10.1002/cncr.32340. PMID: 31290996.

Patents: All patents were filed by the University of Florida Office of Technology Transfer.

Tran DD, Chen D. "*TTFields Induces Immunogenic Cell Death and Sting Pathway Activation through Cytoplasmic Double-Stranded DNA in Glioblastoma Cells.*" UF Disclosure T17793, May 2019. US Provisional Application pending.

Tran, DD, Chen D, 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, Tran DD. "*AAV Capsid Variants Targeting Human Glioblastoma Stem-Like Cells.*" UF Disclosure 190037, February 2019. US Provisional Application planned. (Exclusive licensing agreement to Lacerta Therapeutics, April 2019.)

Le S, Tran DD. "*Core Master Regulators of Glioblastoma Stem Cells*." US Provisional Patent Application filed February 2019: Serial No. 62/802,554.

Le S, Tran DD. "*Methods for Targeted Treatment and Prediction of Patient Survival in Cancer*." US Provisional Patent Application filed February 2019: Serial No. 62/802,653.

Le S, Tran DD. "*Methods for Cancer Screening and Monitoring by Cancer Master Regulators Markers in*

Liquid Biopsy." US Provisional Patent Application filed February 2019: Serial No. 62/802,620.

2. Grant #6BC06: Stress Management Effects on Affect and Influenza Vaccine Response in Older Breast Cancer Patients

Principal Investigators: Michael H. Antoni, PhD, and Bonnie B. Blomberg, PhD, co-PIs

Organization: University of Miami

Progress Report: This study uses a novel technology to deliver stress management interventions to older distressed breast cancer (BCa) patients through a broadband connection. It is intended to show for the first time the efficacy of cognitive behavioral stress management (CBSM) for improving immunological responses to the influenza vaccine (IV) as well as psychological status and inflammatory markers during active primary treatment for BCa. This addresses a major barrier in care: structured interventions delivered in an institutional setting are not feasible for patients due to physical, logistical and acceptability barriers. Because these interventions have mostly demonstrated efficacy using a group format, the study will move the field forward by employing technological advances allowing group-based interventions to be delivered in the home. The intervention occurs before the start of adjuvant therapy, a period of marked anxiety and a "moment of opportunity" when patients are motivated for change. This is the first study to test the effects of stress management on responses to the IV and effective and immune/inflammatory processes in a randomized controlled trial using a remotely delivered group CBSM intervention for older distressed women undergoing treatment for BCa. Over the past year, researchers formally added a breast cancer surgeon as co-investigator; broadened recruitment sites to include Jackson Memorial Hospital (JMH); conducted weekly reviews of patient census at breast cancer clinics at the University Miami/Sylvester Comprehensive Cancer Center and its satellites and JMH; and presented the study at tumor boards, cancer center retreats and national meetings. All of these activities are designed to make the study available to as wide a population of South Florida BCa patients as possible.

During this period, researchers have accrued cases, executed the study protocol including conducting baseline assessments, randomizing cases and deploying the intervention conditions, collected follow-up data, processed samples and conducted quality control monitoring of the data collection. All enrolled participants completed baseline assessments and were assigned to receive either the immediate CBSM intervention condition or the wait-list control (WLC). The online assessments have been successful. Participants can log on using their computers and complete the psychosocial assessment battery. The system is accurately transferring their responses to data files. The CBSM intervention was successfully delivered over the study tablets. Weekly live supervision (aided by videotaped capture of CBSM sessions) ensured that interventionists maintained high fidelity, which was recorded on fidelity monitoring forms.

Based on in-depth ratings of therapist behaviors, the intervention is well executed and well received. Participants have been able to use the tablet-driven system with limited coaching, and any technical challenges were addressed promptly. Participants have successfully received the IV and provided blood samples to assess immune responses at multiple time points. No statistical analyses have been conducted due to ongoing accrual. However, the study design and methods have been presented in multiple venues including tumor boards, cancer center retreats and national/international conferences. They appear on the PI's website for the Sylvester Comprehensive Cancer Center, which recently received National Cancer Institute (NCI) designation. Descriptions of the project have appeared in articles published in referenced journal articles during the reporting period.**Follow-on Funding:** None at the time of reporting.

Collaborations: Three graduate students in the Clinical Health Psychology training program at the University of Miami are receiving training under this research project, though their funding is supported elsewhere: Chloe Taub, Molly Ream, and Erica Nahin. Michael H. Antoni, PhD, a faculty member in the Department of Psychology at the University of Miami, is also performing research under this project. Dr. Antoni brings expertise in empirically evaluating stress management interventions in cancer patients and psychoneuroimmunologic processes.

Two faculty members from the Department of Microbiology and Immunology at the University of Miami are participating in this research project: Bonnie Blomberg, PhD, professor (Co-PI of this grant), and

Daniella Frasca, PhD, assistant professor. Dr. Blomberg brings expertise in the study of cellular and humoral immune functioning and the effects of aging on inflammation in healthy and medical illness patients. Dr. Frasca brings expertise in the use of the influenza vaccine model in animal and human studies on aging with special expertise in examining the role of inflammatory and B-lymphocyte responses.

Marc Lippman, MD, an active emeritus professor of medicine and psychiatry and behavioral sciences at the University of Miami School of Medicine and a professor of medicine at Georgetown University, is performing research for this project. Dr. Lippman is a practicing breast oncologist, basic and clinical researcher, and brings expertise in the study of depression and inflammation in breast cancer patients. Dr. Lippman continues his involvement by consulting without compensation due to his relocation to Georgetown University.

Dr. Susan Kesmodel, an associate professor of surgery at the University of Miami School of Medicine and director of the breast surgical oncology at Sylvester Comprehensive Cancer Center (SCCC), is performing research under this project. Dr. Kesmodel leads a group of breast surgeons at SCCC where she has been charged with increasing the involvement of faculty in research protocols. Dr. Kesmodel joined the study as a co-investigator in December 2017 and began receiving compensation for her involvement shortly after the departure of Dr. Lippman from the university. She meets regularly with the PI and project management on recruitment matters.

The Department of Psychiatry at the University of Miami School of Medicine has had one faculty member performing research under this grant. Professor Sara Czaja, PhD, previously the director of the University of Miami Center on Aging, brings expertise in the use of telecommunications technology (e.g., telehealth) to adapt behavioral interventions for older populations. Dr. Czaja continues her involvement by consulting without compensation due to her new position at Cornell University as director of the Center on Behavior and Aging. In addition to Dr. Czaja, Dr. Dolores Perdomo is a faculty member in the Department of Psychiatry who is performing research.

Journals: Antoni MH, Dhabhar F. Impact of Psychosocial Stress and Stress Management on Immune Responses in Cancer Patients. *Cancer*. 2019;125:1417-1431. PMID: 30768779.

Reis J, Antoni MH, Travado L. Emotional distress, brain functioning and biobehavioral processes in cancer patients: a neuroimaging review and future directions. *CNS Spectrums.*2019;23:1-22. doi:10.1017/S1092852918001621.

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 this research is to quantify early markers of lung radiation toxicty both to lung tissue and pulmonary vasculature and relate these to blood markers of tissue inflammation, clinical signs of loss of respiratory function and patients' quality of life. The 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 armpit and under the sternum. Using tools developed in the research team's lab to extract tissue and vessel response from chest computed tomography (CT) scans, reseachers are able to quantify the severity of damage and 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. The team has now completed analysis of six patients with a minimum of one-year follow-up and has for the first time documented a benefit of proton therapy over conventional X-ray therapy in regards to lung tissue damage. Researchers are continuing to enroll subjects into this study. Thirty subjects have been enrolled to date with an expected 40-plus to be enrolled by the end of the revised recruitment period of

next March. Scans, blood samples, clinical pulmonary function tests and quality of life surveys will be acquired for two years after post-radiation treatment. New, more patient-specific models of tissue response are being formulated to allow for more personalized assessment of a patient's risk and long-term quality of life based on the patient's 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 Univeristy of Florida to pursue independent research projects around this topic.

Research staff also has begun to explore the related issue of 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, early results show for the first time a consistent decrease in global heart function in patients receiving conventional X-ray therapy but a uniform improvement in heart function in proton therapy patients at matched time points post-treatment.

Notably, these changes are measureable using the reserchers' method several years prior to when clinical manifestation of heart damage is expected to occur. This suggests that we are not only able to differentiate response by modality, but also identify patients with the most severe response at early time points when internventions are possible to prevent the patient from experiencing full heart failure.

Follow-on Funding: None at the time of reporting.

Collaborations: Shruti Siva Kumar continues to act as the lead graduate assistant for this project. She is a PhD student in the Department of Biomedical Engineering at the University of Florida (UF) whose efforts are covered by this award. Brandon Terracino is a medical physics PhD student at UF, and his thesis work is gender differences in lung radiation toxicity, 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. Aren is currently funded by 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. Siri Ravuri is a UF undergraduate pre-med student who is analyzing pulmonary vascular changes in a rat model of pulmonary vascular disease, using the same software tools developed for the current award. Siri was awarded a UF Cancer Center University Scholars Program Award for this work. The rat modeling work that Siri is working on involves collaboration with Dr. R. James White and Deborah Haight from the University of Rochester. It is hoped that a new grant application to extend this work will be submitted in the fall of 2019.

The research team's recent abstract submissions include efforts of new early-career radiation oncology clinicians at the UF: Drs. Natalie Lockney, Ray Mailhot and Michael Rutenberg. It also includes new team members medical physicist Xiaoying Liang and nursing PhD student Michele Pembroke.

Journals: None at the time of reporting,

Patents: None at the time of reporting.

APPENDIX E

FISCAL YEAR 2018-2019 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	Haura, Eric	$ 1,686,887	$ 1,256,730.81	$ 430,156.19	5/25/2015	5/15/2020	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: H. Lee Moffitt Cancer Center and Research Institute

Grant Progress Report: The overarching goal of this research project is to utilize signaling-associated complex assays to interrogate aberrant oncogenic signaling in lung cancer. Proteins in cancer cells do not function in isolation, and there are limited methods available to understand how proteins interact in functional complexes to drive growth of cancer cells. Using an innovative approach to study signaling complexes, the research staff generated proof-of-principle data that signaling complexes may represent an entirely novel class of predictive biomarkers. Assessing levels of signaling complexes in lung cancer patient tissues may help in identifying targetable signaling activity and could be harnessed to enable personalized medicine. Tests have been developed that identify signaling complexes for epidermal growth factor receptor (EGFR) and mesenchymal-epithelial transition (MET), two proteins found on the surface of lung cancer cells that can provide pro-growth signaling. There are currently FDA-approved drugs that are active against EGFR and MET, and data acquired as part of this project indicates that high levels of EGFR and MET signaling complexes correlate with sensitivity to these drugs. This work was featured in Clinical Cancer Research in 2017 and in PLoS Biology in 2018.

A novel dual antibody against EGFR and MET is currently being tested as part of a collaboration with Janssen Pharmaceuticals, which has a Phase I trial at Moffitt Cancer Center and other locations. Initial findings, reported at the American Society of Clinical Oncology annual meeting this year, are encouraging with several anti-tumor responses observed in patients who currently have limited therapeutic options. Over the last year, collaborative efforts with Ventana have continued, leading to the no-cost acquisition of a tissue autostainer dedicated to these studies (list price: $250,000). A grant award through the National Cancer Institute's new UH2/UH3 mechanism was recently activated (July 1, 2019), which will enable the continuation of these efforts and will provide three years of funding totaling nearly $1 million in direct costs.

Bankhead-Coley funding has enabled Moffitt Cancer Center to become a recognized leader in the characterization of signaling complexes in tumor tissues, and numerous fruitful collaborations have developed over the course of the project. Research staff has made significant progress toward completion of the scientific aims for the project over the last calendar year and in the final year of the grant are well-positioned to finish aims and continue the established lines of investigation.

Follow-on Funding: National Institutes of Health/National Cancer Institute; Validation of EGFR Protein Complexes as Molecular Diagnostics; Eric B. Haura, MD; $993,537.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

APPENDIX F

FISCAL YEAR 2018-2019 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
8BC11	Dept. of VA	Perez-Stable, Carlos	$ 57,500	$ 57,463.52	$ 36.48	4/12/2018	9/30/2018	No	No	No

1. Grant # 8BC11: A New Strategy to Increas4 Proteotoxic Cell Death in Prostate Cancer

Principal Investigator: Carlos Perez-Stable

Organization: Department of Veterans Affairs

Grant Progress Report: Our progress in the reporting period is in aim 1 "identify potential mediators that link ER stress/unfolded protein response activation to apoptotic cell death in CRV + carfilzomib (Cfz) or ixazomib (Ixz) treated CRPC". We used the LNCaP androgen-dependent PCa and 22Rv1 CRPC cell lines and focused on sub-aim 1-3 "determine the effect of CRV + Cfz/Ixz on pro-survival proteins in CRPC". Effect of CRV + Cfz on ER stress response proteins. The hypothesis of this proposal is the combination of cyclophilin, and proteasome inhibitors will amplify proteotoxic stress, overwhelm the unfolded protein response pro-survival pathway, and force PCa/CRPC cells towards apoptotic cell death without killing normal cells. Proteasome inhibitors also trigger autoohaav. an adaptive pro-survival homeostatic system that helps clear polubiquitinated (Ub) proteins and damaged organelles (1,2). Our initial western blot analysis in LNCaP and 22Rv1 treated with control, CRV (1 pM), Cfz (2 5 nM), and CRV + Cfz for 24 and 48 hours. As a positive control for ER stress response, we used thapsigargin (TG; 5 p,M, 24 h) treated cells. The results showed the expected effects on the apoptosis marker cl-PARP and the autophagy marker LC3 BII, although differences between LNCaP and 22Rv1 were noted.

An important adaptive pro-survival mechanism of unfolded protein response is to reduce proteotoxic stress by the activation of ER localized PERK signaling to increase phospho (P)-elF2a and stop further protein synthesis (3). Results showed modest effects on P and total (T) !F2g. although there appeared to be some elevation of P-elF2a at48 h in both CRV + Cfz treated LNCaP and 22Rv1. This may suggest that the CRV + Cfz combination blocks protein synthesis starting at 48 h (cell death is highest at 72 h). Surprisingly, there were no differences in the ER stress marker GRP78, including in TG treatment. Effects on CHOP (pro-apoptotic ER stress transcription factor) varied. In LNCaP, no induction of CHOP was observed in CRV + Cfz (TG increased CHOP) whereas in 22Rv1, CHOP was induced with CRV + Cfz.CRV + Ixz in LNCaP and 22Rv1.Ixz is a new orally bioavailable proteasome inhibitor with a better tissue distribution compared to Cfz (4).Results with the CRV + Ixz combination in LNCaP and 22Rv1 cells were like CRV + Cfz, i.e., increased cell death, cl-PARP, and poly-Ub compared to either alone. Further increasing CRV from 1 to 2 pM in combination with Ixz increased apoptotic cell death (inhibited by pan-caspase inhibitor QVD) in NCaP and 22Rv1 cells.This may reflect that higher doses of CRV are required for greater efficacy because cyclophilins (CypA, B) are highly expressed in PCa cells (not shown). These results suggest that Cyps are viable targets for PCa chemotherapy

As expected, western blot analysis showed that the CRV (2 pM) + Ixz combination increased cl-PARP at 24 and 48 hours in LNCaP and 22Rv1, which was blocked by QVD caspase inhibitor Differences were noted in LC3BII and poly- Ub. Initial analysis on androgen receptor (AR) in LNCaP (not yet done in 22Rv1) were interesting. The CRV + Ixz combination at 48 h appeared to prevent the recovery of AR protein compared to Ixz and CRV/Ixz/QVD. We are pursuing combinations of CRV/Ixz with the FDA approved AR antagonist enzalutamide and the results are promising (not shown). Effect of CRV + Ixz on ER stress response proteins. The unfolded protein response is an adaptive pro-survival mechanism to ER stress that activates kinase signaling pathways (PERK, IRE1a, and ATF6) to stop further protein synthesis (by increasing P-elF2a) and increasefolding capacity (by increasing CHOP/ATF4 positive transcription factors for chaperones) (3). Our initial western blot analysis of the effects CRV + Ixz on PERK and IRE1a in LNCaP. The results suggested that CRV + Ixz and Ixz alone reduced total (T) PERK levels at 48 h (some differences in ratio of P/T-PERK). Interestingly, QVD blocked the T-PERK decrease. In contrast to PERK, CRV + Ixz and Ixz alone increased T-IRE1a, which was not affected by QVD. At 48 h, T-IRE1a decreased (compared to 24 h), which was blocked by QVD. The ratio of P/T-IRE1a was not affected. XBP1s is downstream of IRE1a and is an activate transcription factor that increases synthesis of

chaperones to counteract proteotoxic stress. Interestingly, at 24 hours, CRV increased and Ixz decreased XBP1s; CRV + Ixz had little effect. At 48 h, XBP1s appeared to decrease greater in CR+Ixz compared with Ixz alone. We tentatively conclude that CRV + Ixz reduces the pro-survival pathways of the unfolded protein response by decreasing PERK, IRE1a, and XBP1s, thus resulting in greater apoptosis and cell death.

SAR405, a new autophagy inhibitor, blocks CRV +Ixz cell death in 22Rv1. Sub-aim 1-2 will "determine the effect of inhibitors of poly-Ub and autophagy signaling pathways on CRV + Cfz or Ixz cell death in CRPC cells". Our initial analysis determined the effects of SAR405, a new and specific inhibitor of Vps34/autophagy (5), on CRV + Ixz in 22Rv1 cells. The results showed that 0.5 (M SAR405 blocked cell death. Surprisingly, SAR405 had only a modest reduction of cl-PARP and LC3BII. We suggest that activation of autophagy may be an important pathway for increasing CRV + Ixz cell death in 22Rv1 but the mechanism is not yet known. In contrast to 22Rv1, SAR405 had no effect on CRV + Ixz in LNCaP perhaps reflecting low induction of LC3BII/autophagy

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journal: None at the time of reporting

Patents: None at the time of reporting

APPENDIX G

FISCAL YEAR 2018-2019 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
6BC01	University of Florida	Ishov, Alexander	$ 100,000	$ 99,438.67	$ 561.33	3/18/2016	8/31/2016	No	No	No
6BC02	University of Miami	Capobianco, Anthony	$ 1,343,732.02	$ 1,233,879.30	$ 109,852.72	4/20/2016	3/31/2019	Yes	No	No
6BC03	University of Florida	Liao, Diaqing	$ 100,000	$ 99,250.87	$ 749.13	3/08/2016	2/28/2017	Pending*	Yes	No
6BC05	Mayo Clinic Jacksonville	Thompson, Aubrey	$ 1,064,624.44	$ 977,589.31	$ 87,035.13	4/12/2016	3/31/2019	No	Yes	No
6BC07	University of Florida	Kladde, Michael	$ 681,887.44	$ 626,141.81	$ 55,745.63	3/31/2016	3/31/2019	Yes	Yes	Yes
6BC08	H. Lee Moffitt Cancer Center	Cleveland, John	$ 1,329,860.86	$ 805,738.01	$ 524,122.85	4/15/2016	3/01/2019	Yes	Yes	Yes

*Received notification of patent submitted but has not yet been finalized.

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

Principal Investigator: Anthony Capobianco, PhD

Organization: University of Miami

Grant Progress Report: During this reporting period, research staff identified the compound NADI-351 as a potent small molecule inhibitor of the Notch transcriptional activation complex through a combination of structure-activity relationship studies (derived from the most potent compound before the above-mentioned period, NADI-260), molecular docking simulations, and *in vitro* studies. The data indicate that NADI-351 inhibits growth of Notch-dependent cell lines, as well as Notch-dependent tumor growth of patient-derived xenograft models of esophageal adenocarcinoma. This was evident by the decrease of Notch target genes expression Notch1 and Hes1 and no effect on the housekeeping gene TBP.

The studies showed that treatment of mice with NADI-351 does not affect mice weight significantly and results in no observable changes in general appearance. Furthermore, unlike gamma secretase inhibitors, NADI-351 does not induce goblet cell metaplasia even at 4 times the efficacious dose used in mouse experiments. This, therefore, validates NADI-351 as a bone fide clinical candidate to move forward into preclinical development.

The research team has also advanced the molecular mechanism of action (MoA) for NADI-351 to gain a better understanding of its more efficacious but less toxic effects. The researchers reasoned that NADI-351 was less toxic because it only inhibits Notch1 complexes and not the related Notch2, Notch3, and Notch4 complexes. In fact, researchers found that treatment of cells with NADI-351 inhibits Notch1 recruitment to the Notch ternary complex, chromatin on HES1 promoter, whereas no effect was observed on the recruitment of Notch2 and Notch3 proteins. These preliminary data suggest NADI-351 selectively inhibits Notch1 recruitment to Hes1 promoter, which has not been previously observed in other Notch inhibitors. Since esophageal adenocarcinoma cell lines, such as OE33, do not express Notch4 protein, researchers used the human breast adenocarcinoma cell line MDA-MB-231 (basal, triple negative breast cancer) that expresses all four Notch receptors (Notch1-4) to determine the effect of NADI-351 on the recruitment of Notch4 to the Notch ternary complex. Treatment of these cells with NADI-351 also inhibits Notch1 recruitment to the Notch ternary complex in a dose-dependent manner but does not affect Notch4 recruitment to the complex. These data indicate that NADI-351 selectively inhibits recruitment to the Notch ternary complex, but no effect is observed for the other Notch paralogs Notch2-4, explaining the superior specificity and efficacy observed for NADI-351 compared to the GSI class of inhibitors. Interestingly, the research team went back into the precursor molecules for NADI-351 and assayed them for selectivity. It discovered several classes of inhibitors, some of which appear to inhibit both Notch 1 and 2 and some only Notch 3. The team is now exploring this in more detail to understand the chemical interactions with Notch and CSL to further exploit this model. This is the first demonstration of Notch selectivity and will be the focus of a new study. Researchers have also now tested NADI-351 efficacy on several distinct patient-derived and tumor cell line xenograft models, including esophageal, lung, breast, and prostate and found that NADI-351 dramatically inhibits the growth of all these tumors.

Collaborations: Rhett Kovall, PhD, an associate professor of molecular genetics in the Department of Molecular Genetics, Biochemistry and Microbiology at the University of Cincinnati College of Medicine, and his team are working in the elucidation of the crystal structure of protein-inhibitor complexes and analysis of biomolecular interactions. His team is currently optimizing conditions for co-crystallization of proteins from the Notch ternary complex with the compounds. As part of their optimization process, Dr. Kovall and his team have obtained new CSL-ANK fusion crystals from Hauptman-Woodward high-throughput screening. These new fusion crystals still need to be optimized and checked for diffraction

before soaking with the selected compounds, including NADI-351. In addition, Dr. Kovall's group is helping to characterize the protein-compound interactions and is planning to perform fluorescence polarization (FP) experiments and isothermal titration calorimetry (ITC) with the constructs. Upon successful completion of these experiments, the team will proceed to titrate in NADI-351 and other selected compounds from the structure-activity relationship study to confirm inhibition of mastermind-like proteins (MAML) recruitment to CSL-ANK fusion construct.

Collaborations: One graduate student (Ellen Kolb) is receiving training under the direction of Dr. Kovall at the University of Cincinnati.

Follow-on Funding: None at the time of reporting.

Journals: None at the time of reporting.

Patents: Capobianco et al. *Inhibitors of the Notch Transcriptional Activation Complex and Methods*. U.S. Provisional Patent Application Filed April 11, 2019. Univ. Miami Reference: UMIP-329. MG Reference: 32286/52683 US.

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

Principal Investigator: E. Aubrey Thompson, PhD

Organization: Mayo Clinic Jacksonville

Grant Progress Report: The research team's analyses have focused on identification of factors that impinge upon diagnosis, prognosis, and treatment of that subset of breast cancers that contain very high levels of the human epidermal growth factor receptor 2 (HER2), known as HER2-positive (HER2+) breast cancer. Research staff cite two major clinical accomplishments during the reporting period.

First, the research team has explored the relationship between patient age at diagnosis, the immune status of the patient, and clinical outcome following HER2-targeted therapy with the monoclonal antibody trastuzumab (Herceptin). The team initially observed that although overall immune status tends to decline as a function of age, the immune landscape of HER2+ tumors does not change. Thus, an age-associated decrease in immune function was not observed at the level of the tumor microenvironment. Consistent with other types of breast cancer, staff observed that the prognosis for HER2+ patients is generally worse for younger patients (<50 years of age); and tumors diagnosed in younger women tend to be more aggressive. Recurrence rates in younger women who receive conventional chemotherapy are greater than those observed in older women similarly treated. However, research staff determined that Herceptin overcomes age-related risk of recurrence in HER2+ breast cancer. Breast cancer-free survival among younger and older HER2+ patients who receive Herceptin is essentially the same.

Second, and perhaps most significant from a clinical standpoint, the research team studied the risk of late recurrence (after 5 years) in HER2+ patients in which the tumor expresses the estrogen receptor (ER). HER2-negative (HER2-) tumors that express ER (HER2-/ER+) exhibit a significant rate of recurrence beyond five years after diagnosis, and current medical opinion favors the idea that such patients should receive ER-targeted endocrine therapy for 15 or even 20 years. Although long-term endocrine therapy decreases the risk of recurrence in HER2-/ER+ patients, such prolonged endocrine therapy is associated with increased costs, potential cardiovascular risk, and a low level of patient compliance. Moreover, it is not known if long-term endocrine therapy would be of benefit in ER+ tumors that express HER2 (HER2+/ER+). The team analyzed HER2+ tumor samples from two large clinical trials to determine if there is a substantial risk of late recurrence in HER2+/ER+ versus HER2+/ER- patients. The data indicate that long-term risk of recurrence in HER2+ tumors is not increased by

expression of ER, in contrast to HER2-/ER+ tumors, which exhibit a significantly increased risk of recurrence compared to HER2-/ER- tumors. The data suggest that endocrine therapy is not required beyond 5 years for HER2+/ER+ patients. This is potentially a “practice-changing” observation.

Collaborations: Pre-doctoral candidate Asleh Karama, Genetic Pathology Evaluation Centre, University of British Columbia, Vancouver, BC

Follow-on Funding: None at the time of reporting.

Journals: Chumsri S, Serie 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; Effect of age and immune landscape on outcome in HER2-positive breast cancer in the NCCTC N9831 (Alliance) and NSABP B-31 (NRG) trials; *Clin Cancer Res.* 2019 Jul 15;25(14):4422-4430. doi: 10.1158/1078-0432.CCR-18-2206. PMID: 30808774. PMCID: PMC6634998 [Available on 2020-07-15].

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: Human genes are regulated by epigenetic mechanisms that include wrapping deoxyribonucleic acid (DNA) around core histones to form nucleosomes. Nucleosomal DNA and histones are subject to further epigenetic regulation via small chemical groups such as methylation. Cancers are frequently caused by changes in nucleosome location and DNA hypermethylation that collaborate to silence tumor suppressor genes. Knowledge of the order of these aberrant epigenetic changes would empower researchers to devise means for earlier cancer detection and hence more effective treatment. This project tests the central hypothesis that alterations in nucleosomes precede DNA hypermethylation in epigenetic silencing of tumor suppressor genes. The temporal order of changes in the locations of nucleosomes and DNA methylation have been determined at different stages of gene silencing in breast cancer in response to an introduced tumorigenic copy of oncogene Harvey rat sarcoma virus (*HRAS*). The researchers have determined epigenetic changes in response to *HRAS* across four cell lines that model human breast cancer progression (“M series;” Karamanos Cancer Institute). M1 cells are non-tumorigenic, immortalized human mammary epithelial cells. Introduction of oncogenic *HRAS* into M1 cells produced pre-malignant line M2, which yielded breast tumors that are non-aggressive (M3) and aggressive/metastatic (M4) in mice. Using M1-M4 cells, Dr. Rosha Poudyal and Dr. Anqi Wang performed Methylation Accessibility Protocol for individual templates (MAPit), an assay developed in Dr. Kladde’s laboratory that simultaneously detects positions of nucleosomes, transcription factors, and DNA methylation. In collaboration with Dr. Nancy Nabilsi (Kappa Biosystems), the MAPit samples were processed by Sequence Capture Epigenetic enrichment of >5.5 million 5'-C-phosphate-G-3' (CpG) sites, a “giant” number (SeqCap Epi CpGiant).

This MAPit-CpGiant approach constitutes the most comprehensive and cost-effective epigenetic assay available to researchers to date and allows further characterization of the aberrant epigenetic insults of the *HRAS* oncogene. A total of 14 MAPit-CpGiant libraries have been sequenced and analyzed using a bioinformatics pipeline developed in-house, DMR2, to identify the differentially methylated and accessible regions across M1-M4 cells. Results were integrated with previously analyzed ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) and published datasets of changes in M series gene expression. Several key tumor suppressor genes and pathways have been identified with epigenetic alterations. These identified genes will be verified during *de novo* expression of oncogenic

HRAS in M1 cells to test the hypothesis that changes in nucleosomes precede DNA methylation. Dr. Wang has introduced HRAS oncoprotein into pre-malignant M1 cells on a retrovirus vector and collected samples for gene expression and epigenetic features tests over a course of 3 weeks. The aim is to capture stepwise intermediates that accompany the transition from active to epigenetically silenced transcription. To do so, research staff has developed an improved and patentable MAPit-NGC (Next-Generation Capture) assay that can assay hundreds of genes from multiple samples at once. All of these molecular analyses have been supported by the development of novel bioinformatics resources. This research will increase the fundamental understanding of the progression of breast cancer and could lead to the use of altered nucleosome occupancy as a novel early biomarker of breast cancer.

Collaborations: The following are receiving training or performing research under this research project:: undergraduate students Williams Owens and Lauren Sevilla, Department of Biochemistry and Molecular Biology, University of Florida, Gainesville; Alberto Riva, PhD, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville; Rhonda Bacher, PhD, Department of Biostatistics, University of Florida, Gainesville; Jonathan D. Licht, MD and Daphné Dupéré-Richer, PhD, Department of Medicine, University of Florida, Gainesville; Christopher D. Vulpe, PhD, Department of Physiological Sciences, University of Florida, Gainesville; Nagi Ayad, PhD, Sylvester Comprehensive Cancer Center, University of Miami.

Follow-on-Funding: University of Florida Genetics Institute; Epigenetic Driven Innate Immune Dysfunction in the Elderly With Sepsis; Phillip Efron, MD - $35,000

National Institutes of Health (NIH)/National Institute of Allergy and Infectious Diseases; *Immune Function and the Progression to Type 1 Diabetes*; Mark A. Atkinson, PhD - to Kladde 5% salary (Year 3) and 7.5% salary (Years 4-5); supplies to be negotiated

DoD, Defense Threat Reduction Agency; *Development of Brassica as a Low Dose Radiation Biosensor*; Patrick Concannon, PhD - $1,392,993

DoD, USAMRMC; *Epigenetic Markers for Susceptibility and Recovery from Exertional Heat Stroke*; Thomas Clanton, PhD – Still in negotiation (funds requested: 1,639,254)

NIH/National Institute of Diabetes and Digestive and Kidney Diseases; *The Role of DNMT3A Mutations in Clonal Heterogeneity & Evolution of Hematopoiesis*; Olga Guryanova, PhD - $1,715,265

Florida Academic Cancer Center Alliance (FACCA); *Epigenetic Basis of Glioblastoma Chemoresistance*; Michael Kladde, PhD - $100,000

NIH/National Cancer Institute; *Vitamin B12 Reprograms Gut Epithelium-Epigenome Resisting Polyposis*; Michael Kladde, PhD, Mansour Mohamadzadeh, PhD - $419,375 (pending)

NIH/National Institute of Aging; *The Pathophysiology of CCI In Septic Older Adults*; Phil Efron, MD - $11,382,898 (pending)

Journals: None at the time of reporting.

Patents: Nabilsi NH, Pardo CE, Kladde, MP. *Determination of Methylation State and Chromatin Structure of Target Genetic Loci.* The University of Florida. International application PCT/US14/773,826 filed September 9, 2015. Notice of Allowance received (awarded for claims 55-60, 63-67, and 71-75) on May 22, 2019. Patent will be issued after UF pays the USPTO fee.

Zhou M, Nabilsi NH, Kladde MP. *Methods and Kits for Targeted Cleavage and Enrichment of DNA for*

Genetic and Epigenetic Analyses. The University of Florida. International PCT application, in preparation.

4. Grant # 6BC08: Epigenetic Regulation of Androgen Receptor in Castration Resistant Prostate Cancer

Principal Investigator: John Cleveland, Ph. D

Organization: H. Lee Moffitt Cancer Center & Research Institute

Grant Progress Report: ACKI is an important non-receptor tyrosine kinase that is upregulated in 25% of prostate adenocarcinomas. Elevated expression of ACK-I positively correlates with advanced stages of prostate cancer (PCa) stages and connotes· poor prognosis. Further, ACKI has been well established to play crucial roles in PCa pathogenesis by regulating multiple signaling cascades. Accordingly, approaches that suppress ACKI activity have shown potent activity in PCa cell lines and using in vivo models of PCa.

One important aspect of PCa cells is its unique metabolic phenotype, which has become a new area of exploration. However, control of PCa metabolism is poorly understood. We have observed that ACKI suppression by our pharmaceutical inhibitor (R)-9bMS compromises mitochondrial functions in PCa cells. Therefore, we have explored how (R)-9bMS mediated inhibition of ACKl regulates PCa metabolic pathways. We used the YSI analyzer to assess metabolites in media collected from prostate cancer cell lines C42B, LAPC4 and 22RVI, and from the normal prostate cancer cell line, R WPE, cultured in presence and absence of (R)-9bMS for 48 hr. Notably, we observed that (R)-9bMS treatment to all three PCa cell line led to significant increases in the levels of extracellular lactate, glutamine and glutamate when compared with untreated cells. Further, levels of glucose were significantly elevated in media from LAPC4 and 22RVI cells following (R)-9bms treatment, whereas this was not evident in the medium of C42B cells treated with (R)-9bms. Interestingly, normal RWPE cells do not show change in-he-1evels of lactate-in media following (R)-9bMS treatment, yet there-were significant increases in the levels of glutamine, glutamate and glucose compared to untreated cells These observations indicate that ACKI inhibition specifically alters either lactate uptake or promotes lactate efflux in PCa cells. To distinguish these possibilities, we will evaluate the effects of (R)-9bMS treatment on: (i) 13C-glucose and 13C-lactate metabolic flux; (ii) levels of intracellular versus extracellular lactate (determined using mass spectrometry); and (iii) uptake of 14C-lactate. These studies are standard in the Cleveland laboratory. We will also perform a full analysis of the effects of (R)-9bMS treatment on the levels of intracellular and extracellular metabolites in PCa versus RWPE cells.

To assess effects of ACKl inhibition on glucose metabolism Next, we performed seahorse assay. C42B and RWPE cells were cultured in media+/- (R)-9bMS for 24 hr and processed these samples for glucose stress test (GST) and mitochondrial stress test (MST) assays using the Seahorse XF metabolic flux analyzer. Notably, ACK inhibition by (R)-9bMS suppresses mitochondri I activ fY in e42l3 a ells but not normal R WPE cells.

Follow-on Funding: National Institute of Health/ National cancer Institute *Molecular Mechanisms of Castration Resistant Prostate Cancer Recurrence & Therapeutic Strategies*. Mahanjan & Feng, Ph.D $3,300,00

Collaborations: None at the time of reporting

Journal: Mahajan NP, Coppola D, Kim J, Lawrence HR, Lawrence NJ, Mahajan K., Blocade of ACK1/TNk2 to Squelch the Survival of Prostate Cancer Stem-like Cells. *Sci rep.* 2018 Jan 31; 8(1): 1954.

Patent: "Allosteric Inhibitor of WEE1 kinase"- Patent # 62/714,351

APPENDIX H

FISCAL YEAR 2018-2019 COMPLETED 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
5BC04	University of Miami	Hu, Jennifer J.	$ 1,290,000	$ 1,279,250.00	$ 10,750.00	5/25/2015	11/15/2018	No	Yes	Pending
5BC08	Sanford Burnham Medical Research Institute	Perera, Ranjan J.	$ 1,289,948	$ 1,288,688.09	$ 1,259.91	5/25/2015	11/15/2018	No	Yes	Yes

1. Grant #5BC04: Impact of Etiology-Driven Precision Medicine on Reducing Breast Cancer Disparities

Principal Investigator: Jennifer J. Hu, Ph.D

Organization: University of Miami

Grant Progress Report: Significant progress has been made on the project, particularly in-patient enrollment (total N=1,571: 861 controls and 710 breast cancer cases) and biopsy sample collection (total N=552). Full-time staff member has increased screening of study subject enrollment and favorably impacted our research. In addition, we have made significant progress in performing laboratory assays for all three aims for OncoArray genotyping (Aim 1), metabolomics (Aim 2), and somatic mutations (Aim 3).

Aim 1: Genotyping and Triple Negative Breast Cancer (TNBC) Etiology – Using the newly developed Infinium OncoArray-500K BeadChip. During this funding period, we have recruited new patients: genomic DNA has been isolated from whole blood ready for genotyping. We have completed 1,280 DNA isolation and quantity checks. During quantitation process, some samples DNA concentration lower than the required concentration for the OncoArray protocol were identified, so research staff have been optimizing concentration before submitting the samples to the cores for genotyping. We have completed all batches of genotyping data collection.

Aim 2: Metabolomics in TNBC Etiology - For Specific Aim 2, the team has proposed to use frozen plasma samples for metabolomics assay. The team finished the non-targeted metabolic profiling with three combined independent platforms: ultrahigh performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS) optimized for basic species, UHPLC/MS/MS optimized for acidic species, and gas chromatography/mass spectrometry (GC/MS). To minimize batch-to-batch variation, we have competed metabolomics assay of three batches of plasma samples of 48 in each batch (i.e., 18 controls, 10 ER+ cases, 10 ER-/HER2+ cases, and 10 triple negative breast cancer).

Aim 3: Next Generation Sequencing of Somatic Mutations in TNBC - For Specific Aim 3, will use the Illumina TruSight RNA Pan-Cancer panel, which provides a comprehensive analysis of the cancer transcriptome. Targeting 1,385 cancer- related transcripts and genes known to be involved in gene fusions, this approach enables analysis of cancer samples including FFPE tissues and other limited samples. This targeted panel offers: (i) gene expression information, variant calling, and fusion detection with known and novel gene fusion partners; (ii) optimized, low- input protocol for a wide range of sample types including FFPE; (iii) a comprehensive view of cancer pathways; and (iv) economical RNA sequencing (RNA-Seq) on a desktop sequencer.

Using all the samples and clinical data collected from this study, we will develop multiple new studies to investigate tumor genomics and immune landscape simultaneously to identify high-risk profiles and targeted innovative therapeutic strategies to improve minority health and overcome survival disparities. The research team will be investigating this new paradigm using data and sample collected from a large tri-racial/ethnic breast cancer population (N=1,800, 67% minorities) and will develop powerful predictive tools in translating functional genomics to targeted interventions, improve clinical outcomes, and overcome breast cancer survival disparities. The research team will target 600 black or African Americans, 600 Hispanic whites, and 600 Non-Hispanic whites

with existing tumor samples and detailed clinical follow-up data.

Three grants were submitted (pending review) based on the data and samples collected in this study. We will continue to develop new projects for future funding opportunities and conduct long-term clinical follow-up studies.

Collaboration: University of Florida Southeast Resource Center for Integrated Metabolomics (SECIM)

Follow-on Funding: None at the time of reporting

Journal: Hu JJ. Urbanic JJ, Case LD, Takita C, Wright JL, Brown DR, Langefeid CD, Lively MO, Mitchell SE< Thakrar A, Bryant D, Baglan K, Strasser J, Baez-Diaz L, Lesser GJ, Shaw EG. Association between Inflammatory Biomarker C-Reactive Protein and Radiotherapy-Induced Early Adverse Skin Reactions in a Multi-Racial/ Ethnic Breast Cancer Populations. J. Clin. Oncol (2018, in press) (Impact Factor 24.008).

Patents: None at the time of reporting

2. Grant# 5BC08: The Expansion and Upgrade of the Analytical Genomics Core Infrastructure at Sanford-Burnham Medical Research Institute

Principal Investigator: Ranjan J. Perera, Ph.D.

Organization: Sanford Burnham Medical Research Institute

Grant Progress Report: Aim 1: To upgrade the next-generation DNA sequencing and DNA microarray capabilities with high capacity and high-throughput platforms. Research staff are working with the llumina corporation to purchase Next-seq 550 machine and with ThermoFisher the qPCR machine Quant Studio. These new machines will help to provide customers cost-effective sequencing and gene expression qPCR services. New (pending) instruments will be posted on the organization's website so as to make available to other researchers. SCID panel was developed with the collaboration of Johns Hopkins All Children's Hospital to detect severe immune compromised children (SCID) was successful. This is a collaboration between the lllumina corporation and our genomics core. We are working with llumina Corporation to fine tune the protocols and are expecting additional results. Research staff are also working on single cell transcriptomics, epigenetics and hope to use Next-seq 550 to process samples. Inventory (reagents, instruments, protocols, tissue, RNA, and DNA) is well documented in Access and Excel files until a solution is found to acquire the LIMS system.

Collaboration: Dr. Barbara Sharanwski, University of Central Florida; Dr. Carole Perrot, Sanford Burnham Prebys Medical Discovery Institute; Dr. Jolan Walter, University of South Florida; Dr. Bongyong Lee, Sanford Burnham Prebys Medical Discovery Institute

Follow-on Funding: None at the time of reporting

Journal: Marta Diez-Cunado, Ke Wei, Paul Bushway, Mano R Maurya, Ranjan J. Perera, Shankar Subramaniam, Pilar
Ruiz-Lozano and Mark Mercola. miRNAs that induce human cardiomyocyte proliferation converge on the Hippo pathway. Cell Reports (Accepted in Press) 2018.

Yunyun Chen, Jiqiang Yao, Alexey Eroshkin, Niveditha Nerlakanti, Ami Patel, Neha Agarwal, Duy Ngyuen, JianLiang Li, Zhenjun Ma, Subramaniam Shymalagovindarajan, Jamie Teer, Jasreman Dhillon, Ranjan J. Perera**,** Youngchul Kim, and Kiran Mahajan. The Homeobox Gene, HOXB13, Regulates a

Mitotic Protein-Protein Interaction Network Predictive of Metastatic Prostate Cancer. Clinical Cancer Research (in review) 2018.

Xiao-Peng Xiong, Antonio Tito, Jian-Liang Li, Shiyu Xu, Julia Situ, Ranjan J. Perera, Sheng Zhang, and Rui Zhou. The Circular RNA *Edis* Modulates Innate Immunity and Neurodevelopment in *Drosophila.* Nature Communication (in review) 2018.

Kirthana Prabhakar, Ashika S. Jayanthy, Carlos I. Rodriguez, Ranjan J. Perera, Vijayasaradhi Setaluri. Oncogenic functions of miR-214 in melanoma and its role in regulation of p-catenin expression. JBC (in review)
2018.

Patents: None at the time of reporting

King Research Program

APPENDIX I

FISCAL YEAR 2018-2019 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
9JK01	Florida State University	Salazar, Gloria	$ 805,409	$0.00	$ 805,409	9/18/2019	9/30/2022	No	No	No
9JK02	H. Lee Moffitt Cancer Center	Tworoger, Shelley	$ 504,838	$0.00	$ 504,838	8/15/2019	8/31/2022	No	No	No
9JK03	MCI/ Baptist Health South Florida	Calero, Miguel Villalona	$ 1,187,224	$0.00	$ 1,187,224	Pending	Pepnding	No	No	No
9JK04	University of Central Florida	Copik, Alicja	$ 805,409	$0.00	$ 805,409	10/2/2019	9/2/2022	No	No	No
9JK05	University of Florida	Salloum, Ramzi	$ 404,909	$0.00	$ 404,909	10/09/2019	9/30/2022	No	No	No
9JK06	University of Florida	Zajac-Kaye, Maria	$ 805,409	$0.00	$ 805,409	9/18/2019	9/30/2022	No	No	No
9JK07	University of Miami	Dudeja, Vikas	$ 805,393	$0.00	$ 805,393	9/18/2019	8/31/2022	No	No	No
9JK08	University of Miami	Dave, Kunjan	$ 805,409	$0.00	$ 805,409	9/4/2019	9/30/2022	No	No	No
9JK09	University of Miami	Banerjee, Sulagna	$ 805,409	$0.00	$ 805,409	9/24/2019	9/30/2022	No	No	No
9JK10	University of South Florida	Philpot, Rex	$ 771,341	$0.00	$ 771,341	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 A. Salazar, PhD

Organization: Florida State University

Abstract of Proposed Research: Smoking and aging are two major risk factors in cancer and cardiovascular disease (CVD). Although recent reports show that smoking stimulates senescence in the lung, it is unknown whether smoking also accelerates senescence of the cardiovascular system. In this study, researchers propose the novel hypothesis that cigarette smoke and nicotine accelerate vascular senescence, promoting the development of atherosclerosis. The researchers hypothesize that aging and smoking activate a common molecular mechanism that depends in part on the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase NOX1 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.

The research team has 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 induces senescence. Novel preliminary data show that blackberry supplementation reduced senescence and atherosclerosis in apolipoprotein E (ApoE) knockout mice in vivo and that nicotine alone is enough to increase atherosclerosis in the ApoE knockout 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. In this proposal, the researchers will test the hypothesis that inhibition of NOX1 by blackberry polyphenols reduces the SASP, thus diminishing senescence and atherosclerosis caused by tobacco smoke. This hypothesis will be tested through the following aims: 1) 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.

Follow-on Funding: None at the time of reporting.

Collaborations: Hyun Seok Hwang, PhD (Co-Investigator), Florida State University; Bahram Arjmandi, PhD, RD (Co-Investigator), Florida State University; Judy Muller-Delp (Co-Investigator), Florida State University; Kathy Griendling, PhD (Collaborator), Emory University; Dr. Colin Kay, PhD (Collaborator), North Carolina State University; and Pradeep Bhide, PhD (Collaborator), Florida State University.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Shelley Tworoger, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Abstract of Proposed Research: Ovarian cancer is the fifth leading cause of cancer death in the U.S, and the sixth leading cause in Florida. Since most cases are diagnosed at an advanced stage, identifying novel risk factors is crucial to reduce incidence and mortality. Several lines of evidence suggest that early life exposures may be relevant to ovarian cancer risk. Most ovarian cancer risk factors (e.g., parity, oral contraceptive use) occur during childbearing years, suggesting a susceptibility window earlier in life. Also, in the researchers' own data, a larger body size at age 10 was associated with reduced ovarian cancer risk while body mass index during adulthood was associated with higher risk. These data suggest that exposures occurring during a critical period in early life may uniquely influence risk.

The objective of this proposal is to evaluate several early life exposures (cigarette smoking, social adversity and abuse and physical activity) and risk of ovarian cancer in later life. Better understanding of the role of early life factors in ovarian cancer risk may help inform development of targeted prevention strategies. First, current cigarette smoking is associated with a two-fold increased risk of mucinous ovarian cancer tumors. Yet, few studies have examined the potential impact of early life exposure to cigarettes on risk. The researchers will generate novel data on ovarian cancer risk in relation to age at smoking initiation, having a parent that smoked inside the home during childhood and having a mother who smoked during pregnancy.

In addition, in the researchers' own data, a two-fold increased risk of ovarian cancer was observed among women with post-traumatic stress disorder (PTSD) symptoms versus those who did not experience trauma. Early life stress is a common underlying cause of PTSD and other distress disorders in adulthood and has a potential role in altering ovarian development at the time of exposure. Thus, the researchers propose to examine ovarian cancer risk in relation to early life social adversity and abuse. Finally, the research team's initial data suggests higher levels of adult premenopausal physical activity modestly increase ovarian cancer risk while postmenopausal physical activity has no impact on risk. To extend these findings, the researchers propose examining associations with physical activity during middle school, high school, and ages 18-22. This data will help fill in gaps in knowledge about how physical activity may impact risk of ovarian cancer differently over the life course and whether physical activity profiles could be useful in risk prediction.

To conduct these epidemiologic studies, researchers propose to leverage data from the prospective, ongoing Nurses' Health Study cohorts, which enrolled more than 200,000 women across the U.S. Since enrollment, participants have completed biennial questionnaires about their medical history, health behaviors and early life exposures. Ovarian cancer cases are identified by self-reporting and death registry and confirmed by medical record review or cancer registry linkage. For each proposed analysis, researchers will analyze risk overall and by histotype. Secondarily, among cases with archived tumor tissue, they will measure tumor immune cell profiles, allowing assessment of associations with tumors that exhibit an immunosuppressive signature. Overall, the researchers propose to use a unique life course approach to improve identification of women at high risk of ovarian cancer. These findings, together with other known health effects of these exposures in early life, will give further support for implementing novel interventions in this time period.

Follow-on Funding: None at the time of reporting.

Collaborations: Christine Vinci, PhD, Co-Investigator, Moffitt Cancer Center; Jose Conejo-Garcia, PhD, Co-Investigator, Moffitt Cancer Center; Danielle Jake-Schoffman, PhD, Co-Investigator, University of Florida; Mary Townsend, ScD, Applied Research Scientist, Moffitt Cancer Center; and Tianyi Wang, PhD, Post-Doctoral Fellow, Moffitt Cancer Center.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Miguel Villalona Calero, MD

Organization: Miami Cancer Institute, Baptist Health South Florida

Abstract of Proposed Research: Cervical cancer is the third most common gynecological cancer in the U.S., and women who smoke and are human papillomavirus (HPV) positive have up to three times the risk of developing cervical tumors compared to nonsmokers. The incidence remains elevated in the Hispanic population, which also presents with more advanced disease and worse mortality. Notable disparities in this ethnic subgroup correlate with poor access to healthcare and lower socioeconomic status. Hispanics make up the largest ethnic minority in Florida; therefore, the disease is an important public healthcare concern in the state today. A newly approved option in second-line treatment for advanced cervical cancer is the immune checkpoint agent pembrolizumab, an immunoglobulin G4 (IgG4) monoclonal antibody which blocks binding of programmed cell death protein (PD1) to programmed death-ligand 1 (PDL1) and programmed death ligand 2 (PDL2), helping restore T cell immune response. This was based on a very low overall response rate (ORR) of only 13.3%. Therefore, better strategies are needed to increase the efficacy of immune checkpoint blockade. One 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 (PARP) inhibitors, which lead to accumulation of DNA single-strand and consequently double-strand breaks in patients with breast cancer (BRCA)-mutated tumors that are innately deficient in homologous repair (HR). BRCA genes collaborate with several others in the Fanconi anemia (FA) HR pathway, so the research team developed an immunofluorescence-based method: FA, complementation group D2 (*FANCD2)*/4′,6-diamidino-2-phenylindole (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. The researchers screened over 600 patients in a clinical trial and found a functional deficiency in 29% of solid tumors. They also showed it is safe to administer the PARP inhibitor veliparib with the DNA damaging agent mitomycin C to patients with FA deficient tumors.

The researchers 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. They hypothesize that cervical cancer patients will have improved responses to pembrolizumab when given in combination with olaparib, a potent PARP inhibitor, and that the FATSI assay could serve as an indicator of tumor response to immune checkpoint inhibition in FA deficient tumors. In order to support this hypothesis, research staff will address the following research aims: 1) assess the efficacy of the combination of pembrolizumab and olaparib in patients with advanced cervical cancer; and 2) investigate whether functional deficiencies in the FA pathway of cervical cancers will correlate with improved response to the combination.

To accomplish Aim No. 1, the research 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 greater than 35%. To accomplish Aim No. 2 the researchers 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: Ludimila Cavalcante, MD, Miami Cancer Institute, Baptist Health South Florida; Muni B. Rubens, PhD, statistician, Miami Cancer Institute, Baptist Health South Florida; John Diaz, MD, Co-Investigator, Miami Cancer Institute, Baptist Health South Florida; Wenrui Duan, PhD, Collaborator, Florida International University; and Giri Narasimhan, PhD, Collaborator, Florida International University.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

4. Grant #9JK04: Adoptive PM21NK Cells with PDL1 Blockade for Treatment of Lung Cancer

Principal Investigator: Alicja J. Copik, PhD

Organization: University of Central Florida

Abstract of Proposed Research: 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 lower the relapse rate. To achieve the proposed goal, the project will leverage the unique capabilities of ex vivo expanded natural killer (NK) cells reprogrammed to be highly activated through exposure to membrane particles (Particulate Matter 21 [PM21]) or exosomes (EX21) derived from Interleukin 21 (IL21) expressing feeder cells (FCs) K562 membrane-bound IL-21 (mbIL21) 4-1BB ligand (4-1BBL) membrane-bound 21-FCs. These PM21 particles stimulated NK cells to produce Interferon (IFN) gamma in response to encounters with tumor cells to induce programmed death-ligand 1 (PDL1) expression. Induced PDL1 can be then targeted by humanized anti-PDL1 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 immune cells, such as dendritic cells, as well as cytotoxic and helper T cells to further direct complete elimination of cancer.

The researchers hypothesize that this approach has the potential to turn "cold tumors" "hot" to greatly improve treatment outcomes. Their method using nanoparticles (PM21) and exosomes (EX21) derived from mb21-FCs further introduces new therapeutic dimensions by: 1) a feeder cell-free expansion and stimulation system that can produce high NK cell numbers; 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 will test parameters to inhibit the immunosuppressive environment and enhance NK cell antitumor 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 who did not respond to or relapsed on anti-PD1/PDL1 therapy.

Follow-on Funding: None at time of reporting.

Collaborations: Deborah Altomare, PhD, University of Central Florida; Stephen Florczyk, PhD, University of Central Florida

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

Abstract of Proposed Research: Tobacco use and tobacco smoke exposure (TSE) remain the leading preventable causes of mortality and morbidity for families in Florida and nationwide. Tobacco cessation in parents adds an average of seven years to their life, eliminates most of their children's TSE, decreases the odds that children become tobacco users and improves the financial status of disadvantaged families. The pediatric setting presents unique and important opportunities to address parents' tobacco use to reduce TSE in children. The effectiveness of tobacco control strategies in clinical settings is well-established, yet compliance in pediatric practice remains low. Consequently, it remains unclear how to best support the uptake and sustainability of delivering evidence-based tobacco control interventions to parents in pediatric practice. This gap must be filled to inform the development of integrated and sustainable support that will effectively reduce TSE in children and families. The overarching goal of this project is to design clinical support strategies to enhance the delivery of tobacco control interventions in pediatric practice that can be scaled for wider implementation. Training for providers and clinic staff promotes best practices for tobacco control in clinical care, but implementation remains insufficient due to barriers to clinical efficiency, including competing time constraints during an office visit. However, the diffusion of electronic health records (EHRs) into clinical practice increases opportunities to engage clinics in intervention approaches that are potentially more sustainable by capitalizing on existing clinical processes. The researchers propose a two-pronged approach to enhance implementation: 1) training providers and office staff on current best practices; and 2) deploying a brief EHR-based intervention in conjunction with provider-engaged adaptations to fit the intervention into practice workflow. Specifically, the research team will assess the feasibility and efficacy of a scalable, automated EHR tool for tobacco screening and counseling, along with provider training on best practices, among diverse clinics in the OneFlorida Clinical Research Consortium. Using a National Cancer Institute-designated Research Tested Intervention Program (RTIP), the team developed and piloted an innovative EHR-based process that confidentially screens parents pre- or in-visit for the use of tobacco and nicotine products. Tobacco users watch a brief evidence-based tobacco control video tailored to the tobacco product(s) they use, as assessed through the screening process. The doctor receives the screening results in the EHR to enable counseling in addition to an EHR-based referral to cessation services. The researchers will pursue the following aims: 1) determine the feasibility of a parental tobacco control intervention combining EHR and provider training features to implement in a group randomized trial of six clinics. The primary outcome for feasibility is patient reach (i.e., receipt of the intervention), and the secondary outcome will be abstinence from tobacco use at six months; 2) identify the predictors of patient reach among parents who are tobacco users. Potential predictors include patient sociodemographic factors and other health risk behaviors; and 3) measure variability in implementation outcomes and identify whether implementation is moderated by practice and provider factors including provider self-efficacy, practice capacity for change and adaptive reserve, and clinic level patient social characteristics.

Follow-on Funding: None at the time of reporting.

Collaborations: Lindsay A. Thompson, MD, Co-Investigator, University of Florida; Elizabeth A. Shenkman, PhD, CoInvestigator, University of Florida; Ryan P. Theis, PhD, Co-Investigator, University of Florida; Matthew J. Gurka, PhD, CoInvestigator, University of Florida; and Jiang Bian, PhD, Co-Investigator, University of Florida.

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

Abstract of Proposed Research: According to the American Cancer Society, exposure to tobacco products is one of the most important risk factors for pancreatic cancer. For example, smokers have a two-fold excess risk for pancreatic cancer compared to never-smokers. Approximately 25% of pancreatic cancers are thought to be caused by cigarette smoking, and there is evidence implicating cigar and pipe smoking as well as using smokeless tobacco products. There are no effective systemic treatments for advanced pancreatic cancer which is now projected to be the second leading cause of cancer-related deaths in the U.S. by 2020. Surgery provides the only curative therapy for pancreatic ductal adenocarcinoma (PDAC), but less than 20% of patients are suitable candidates due to the challenges associated with detecting 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 (folinic acid, fluorouracil, irinotecan, oxaliplatin) in patients with advanced disease, the survival rate 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.

The researchers propose to investigate novel therapeutics that better exploit the molecular basis of pancreatic cancer. The overall goal of this proposal is to use a newly established animal model to test novel drug compounds in treatment of pancreatic cancer. The research team has established an animal model for pancreatic cancer by generating genetically engineered mice that conditionally express mutant Kirsten Ras (KRAS) and human thymidylate synthase (hTS/TS) in the pancreas. TS, an essential enzyme for DNA synthesis and repair, is aberrantly overexpressed in a range of human cancers including PDAC. Dr. Zajac-Kaye's laboratory demonstrated that overexpression of TS in the pancreas promoted aggressive PDAC development and markedly reduced survival of KRAS mutant mice. TS overexpression is also linked with resistance to gemcitabine and fluorouracil (5-FU) which are the primary chemotherapy treatments for PDAC. Thus, the goal of this proposal is to develop new treatments for pancreatic cancer using unique TS allosteric inhibitors identified in Dr. Zajac-Kaye's laboratory. The researchers' newly developed derivatives do not induce feedback activation of TS levels and are not associated with drug resistance when tested in PDAC tumor cell lines. In addition, preclinical data show that TS inhibitors synergistically enhance RAS/phosphoinositide 3-kinase (PI3K)/*protein kinase B* (AKT)/*mammalian target of rapamycin* (*mTOR)* inhibition in vitro. In this project, the researchers plan to test new TS inhibitors alone or in combination with mTOR inhibitors using this novel in vivo hTS/KRAS PDAC model. In addition, they propose determining the antitumor activity of the same drug combination in human samples using patient derived xenografts (PDX) from smoking and non-smoking associated with freshly collected PDAC biopsies. This research will lay the groundwork for NCI R01 application within three years and a personalized investigator-initiated clinical trial in five years. The ultimate goal is that the innovative targeting of TS and KRAS pathways will provide a new effective strategy for patients with advanced PDAC.

Follow-on Funding: None at the time of reporting.

Collaborations: Jose Trevino, MD, University of Florida; Frederic J Kaye, MD, University of Florida; Maria Guijarro Barrigon, PhD, Project Personnel, University of Florida; Akbar Nawab, PhD, Project Personnel, University of Florida; Min Zhang, PhD, Project Personnel, University of Florida; Dr. Zhang, Project personnel, University of Florida; and Thomas George, MD, Collaborator, University of Florida.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Vikas Dudeja, MD

Organization: University of Miami

Abstract of Proposed Research: Despite decades of research, the outcomes of pancreatic cancer are largely unchanged. While the pathogenesis of pancreatic cancer is far from clear, smoking is one of the major risk factors for pancreatic cancer. However, the mechanism by which smoking increases the risk of pancreatic cancer, or, for that matter, any cancer is still being unraveled. Recent years have seen an increase in the understanding of the role of gut microbiome in health as well as in the pathogenesis of cancer.

Intriguingly, the researchers' preliminary data suggest that administration of nicotine-derived nitrosamine ketone (NNK), one of the key tobacco-specific nitrosamines, leads to increased pancreatic cancer growth and a remarkable change in the gut microbiome in mice. NNK is commonly used as a surrogate for smoking in studies evaluating pathogenesis of smoking-induced cancers.

Preliminary data also suggest that depletion of gut microbiome with a broad spectrum, poorly absorbable antibiotics cocktail counteracts the growth-promoting effects of NNK. This suggests that the pancreatic cancer growth-promoting effect of NNK is mediated by modulation of gut microbiome. Also, NNK administration leads to decreased infiltration of activated cytotoxic T cells, suggesting that NNK reduces anticancer immune response. Intriguingly, depletion of gut microbiome prevents NNK-induced suppression of anti-cancer immune response. Based on this, the research team hypothesizes that smoking inhibits anticancer immune response by modulating gut microbiome. This novel hypothesis will be evaluated in the current grant proposal.

In Aim No. 1 of the current grant proposal, the research team will confirm the preliminary finding that smoking promotes pancreatic cancer progression through modulation of gut microbiome. For this, the researchers will use a genetically engineered mouse model as well as orthotopic models of pancreatic cancer. The effect of smoking (simulated by use of NNK or smoking chambers) on the tumor growth and progression will be measured with and without gut microbiome depletion (with use of broad spectrum, poorly absorbable antibiotics cocktail). While NNK is one of the most commonly used compounds to recapitulate the effects of smoking, use of smoking chambers more closely recapitulates the effect of smoking as tobacco smoke has more than 7,000 chemicals. Finally, the research team will use fecal transplantation to further confirm the role of gut microbiome modulation on the effect of tobacco smoking on tumor growth and progression.

In Aim No. 2 of the current grant proposal, the researchers will characterize the gut microbiome changes induced by smoking in models of pancreatic cancer. They will use various strategies to elucidate which bacterial community mediates the tumor-promoting effects of smoking.

Finally, in Aim No. 3, the research team will evaluate the immunological changes induced by smoking and establish the role of smoking-induced gut microbiome modulation in these immune changes. These proposed studies will not only elucidate novel mechanisms by which tobacco smoking promotes tumor growth but will also lead to the discovery of novel strategies which can counteract harmful carcinogenic effects of tobacco smoking. For instance, the effect of tobacco smoke-induced microbiome changes can be counteracted with novel strategies (antibiotics vs. probiotics). Furthermore, researchers expect to better understand how cigarette smoking affects immune response against tumor cells as well

as the role of smoking on gut microbiome in modulating immune functions. The combination of expected observations makes this research novel and significant.

Follow-on Funding: None at the time of reporting.

Collaborations: Ashok Saluja, PhD, Co-Investigator, University of Miami; Sabita Roy, PhD, Co-Investigator University of Miami; Eli Gilboa, PhD, Co-Investigator, University of Miami; *Xi* (*Steven) Chen*, PhD, Co-Investigator University of Miami; Monica Garcia, MD, Co-Investigator, University of Miami; Vrishketan Sethi, Project Personnel, University of Miami; and Roey Hadad, Project Personnel, University of Miami.

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

Abstract of Proposed Research: Smoking is one of the main risk factors for spontaneous intracerebral hemorrhage (sICH): the deadliest subtype of stroke. However, the effect of smoking on outcomes following sICH is not known. 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 not able to offer more than supportive care. The prevention of continued bleeding in sICH has been a promising therapeutic target. Dr. Wenche Jy (co-investigator) and his group have studied red blood cell microparticles (RMP) as hemostatic agents for over a decade. Strong preliminary results from Dr. Jy's laboratory have demonstrated that RMP support and enhanced hemostasis at sites of bleeding, RMP are effective in correcting hemostatic defects in both platelet and coagulation disorders. They remain effective in the presence of anti-platelet drugs, and RMP are equally effective in treating microvascular and macrovascular bleeding. The goal of this project is to determine the effect of chronic nicotine exposure on outcomes following sICH and if RMP treatment improves post-sICH outcomes (histological, behavioral and inflammation, among others) in chronic nicotine-treated rats via limiting hematoma volume. Researchers will use preclinical models of autologous blood and collagenase-induced sICH. They hypothesize that chronic nicotine exposure will worsen outcomes following sICH, and RMP will be able to limit hematoma expansion in a clinically relevant animal model of sICH. This hypothesis will be tested in this proposal.

Follow-on Funding: None at the time of reporting.

Collaborations: Sebastian Koch, MD, University of Miami; Yeon S. Ahn, MD, Collaborator, University of Miami; Miguel A. Perez-Pinzon, PhD, Co-Investigator, University of Miami; Ami P. Raval, PhD, Co-I-nvestigator, University of Miami; Hever Navarro Quero, Project Personnel, University of Miami; and Sunjoo Cho, Project Personnel, University of Miami.

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

Abstract of Proposed Research: Tobacco smoking is one of the major risk factors for pancreatic cancer, a disease with very poor survival rates. The poor prognosis of this disease is attributed to the presence of a dense fibro-inflammatory stroma consisting of the extracellular matrix, stromal cells and the infiltrated immune population. This creates a complex tumor microenvironment that is conducive to an aggressive disease that is resistant to therapy, extremely metastatic and prone to recurrence. Studies from this group as well as others have shown that increased expression of pentaspan membrane glycoprotein prominin-1 (CD133) contributes to aggressive biology in pancreatic ductal adenocarcinoma (PDAC). These cells are treatment refractory, extremely metastatic, and contribute to tumor recurrence. Recent studies show that cancer cells undergo dynamic interconversion between aggressive and non-aggressive states as a result of their interaction with the other cells in the microenvironment. This plasticity between an aggressive and non-aggressive phenotype adds to the challenges for developing a viable therapy against pancreatic cancer that can prevent recurrence and overcome therapeutic resistance. Understanding the molecular mechanism of this dynamic interconversion thus holds the key for developing successful therapy against pancreatic cancer.

The research team's previously published study shows that the CD133+ aggressive cells exhibit an altered metabolic profile from the non-aggressive population, which offers them a distinct survival advantage. It is well known that pancreatic tumors have a robust fibro-inflammatory stroma that is extremely reactive. Preliminary data showed that in the presence of the stroma, there is a distinct enrichment of CD133+ cells. Results show that this enrichment is due to the signaling mediated by the secreted cytokine Interleukin 6 (IL6) from the stromal cells. IL6 also contributes to an altered metabolic phenotype in the CD133+ cells that is responsible for their survival advantage and aggressive phenotype. Based on these observations, the researchers hypothesize that the stromal component of the microenvironment promotes aggressive biology and metabolic reprogramming in a population of tumor cells resulting in a resistant phenotype. These metabolically re-wired cells also lead to an immune suppressive microenvironment, thereby resulting in a tumor that is unresponsive to most therapy. The researchers thus propose that targeting the stromal secretion will inhibit this plasticity and overcome therapeutic resistance pancreatic cancer. This hypothesis will be validated by 1) evaluating the role of stromal component in inducing metabolic reprogramming in pancreatic cancer; 2) elucidating the mechanism by which metabolic reprogramming leads to a survival advantage in CD133+ population in PDAC; and 3) whether the stromal secretion can be targeted to overcome plasticity and therapeutic resistance in pancreatic cancer.

Follow-on Funding: None at the time of reporting.

Collaborations: Ashok Saluja, PhD, Co-Investigator, University of Miami; Steven Chen, PhD, Co-Investigator, University of Miami; Kousik Kesh, PhD, Project Personnel, University of Miami; Vikas Dudeja, MD, Co-Investigator, University of Miami; and Vineet Gupta (Postdoctoral Fellow), University of Miami.

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

Abstract of Proposed Research: Smoking is linked to a higher risk of breast cancer in younger, premenopausal women, with some studies indicating as much as a 40% increase in risk. A majority of younger women diagnosed with breast cancer live for several decades following diagnosis and treatment. Therefore, there is a considerable need for research focusing on the long-term quality of life of breast cancer survivors. Chemotherapy-related cognitive deficits (CRCDs) are a common outcome of cancer treatment, occurring in up to 75% of patients. For some, cognition improves after treatment is complete, but impairment in one or more cognitive domains persists indefinitely for more than 50% of those who experience a cognitive deficit. These deficits can persist for more than 20 years following treatment, interfering with daily functioning, the ability to return to work and reducing quality of life. Although many cancer patients experience CRCDs, and these deficits are associated with changes in brain structure and function, the mechanisms underlying the occurrence of these deficits are not understood. There are no treatments approved for this condition. Findings suggest that the manifestation of CRCDs involves reductions in estrogen and/or tumor- and chemotherapy-associated increases in pro-inflammatory cytokines. However, it is unclear how these consequences translate into long-lasting cognitive deficits. The proposed studies will model CRCDs using cyclophosphamide (CYP) and doxorubicin (DOX), agents used to treat breast cancer, to induce deficits in the working, spatial and/or procedural memory of mice with breast cancer (mouse mammary tumor virus- [MMTV]-polyomavirus middle *T antigen mice* [PyVT])*.* This model will be used to investigate the hypothesis that chemotherapy-induced reductions in circulating estrogen renders cholinergic neurons uniquely vulnerable to injury and death in tumor-bearing individuals who receive chemotherapy and that selective muscarinic acetylcholine receptor (mAChR) agonists can be used to prevent, or to treat, CRCDs that persist following chemotherapy.

Aim No. 1 will use tumor-free and tumor-bearing mice to test the hypothesis that CYP+DOX administration reduces cholinergic cell number in the central nervous system (CNS) and assess whether reductions in estrogen or increases in pro-inflammatory cytokines are related to the loss of cholinergic neurons or the manifestation of CRCDs. Aim No. 2 will test the hypothesis that selective mAChR agonists can treat CYP+DOX-induced cognitive deficits in MMTV-PyVT mice and, if these agonists are effective, determine whether these drugs adversely affect tumor growth or the efficacy of the chemotherapeutic agents. Aim No. 3 will mechanistically test the hypothesis that CYP+DOX-induced reductions in high affinity choline uptake in neurons of the central nervous system, decreased acetylcholine (Ach) synthesis and/or impaired regulation of pro- inflammatory cytokines is/are a consequence of chemotherapy-induced reductions in estrogen. Results of these studies will determine whether CRCDs are a consequence of an estrogen-mediated impairment of cholinergic function and whether this impairment is associated with the presence of tumors and/or increases in pro-inflammatory cytokines. In addition, these studies will determine whether selective mAChR agonists can treat CRCDs and evaluate the impact of mAChR agonists on tumor growth and the effectiveness of chemotherapy. These findings will help identify a mechanism for the occurrence of CRCDs and establish mAChR agonists as a viable treatment.

Follow-on Funding: None at the time of reporting.

Collaborations: Paula Bickford, PhD, Co-Investigator, University of South Florida College of Medicine; Bethany Johns, Project Personnel, University of South Florida College of Medicine; Lauren Moss, Project Personnel, University of South Florida College of Medicine; Lynn Wecker, PhD, Collaborator, University of South Florida College of Medicine; Heather Jim, Consultant, Moffitt Cancer Center; and Michelle Janelsins, PhD, Consultant, Wilmont Cancer Institute.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

APPENDIX J

FISCAL YEAR 2018-2019 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
8JK01	Florida Atlantic University	Fields, Gregg	$ 708,044	$ 248,772.00	$ 459,272.00	5/11/2018	4/30/2021	No	Yes	No
8JK02	H. Lee Moffitt Cancer Center	Permuth, Jennifer	$ 1,360,857	$ 453,616.00	$ 907,241.00	5/03/2018	3/31/2021	No	No	No
8JK03	H. Lee Moffitt Cancer Center	Kumar, Nagi	$ 708,044	$ 141,608.00	$ 566,436.00	5/03/2018	3/31/2023	No	No	No
8JK04	University of Florida	Kaye, Frederic J.	$ 1,360,857	$ 232,760.84	$ 1,128,096.16	6/06/2018	3/31/2023	No	Yes	No
8JK05	University of Florida	Kusmartsev, Sergei	$ 816,514	$ 272,172.00	$ 544,342.00	5/04/2018	3/31/2021	No	Pending*	No
8JK06	University of Florida	Hayward, Linda F.	$ 816,514	$ 272,172.00	$ 544,342.00	5/07/2018	3/31/2021	No	No	No
8JK07	University of Miami	Saluja, Ashok	$ 816,514	$ 272,172.00	$ 544,342.00	4/25/2018	3/31/2021	No	No	No
8JK09	University of South Florida	Ghansah, Tomar	$ 816,514	$ 249,497.00	$ 567,017.00	4/17/2018	3/31/2021	Yes	Yes	No

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

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, among 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 several tobacco-related cancers and the collagen-cleaving ability of MT1-MMP is critical to the progression of several 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 used to design novel inhibitors. The specific aims to achieve these goals are as follows: 1) quantitative analysis of MT1-MMP activity on the cell surface, including the modulation of activity by specific MT1-MMP domains and binding partners; and 2) 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 researchers have made progress in three areas. First, the research team completed the expression of five enzymes: full-length MT1-MMP; soluble MT1-MMP (no transmembrane domain); the catalytic domain of MT1-MMP; full-length MMP-8; and the catalytic domain of MMP-8, with the appropriate tags needed for screening against the combinatorial peptoid-inspired, conformationally constrained oligomers (PICCO) library. Full-length MT1-MMP was especially challenging, with a yeast expression system providing the best results. Second, the researchers obtained quantitative results for the cell-based assay using wild-type, full-length MT1-MMP and several mutants. That work has been published. (See below.) The research team determined that inhibitor activity can be quantified using the cell-based assay. The cell-based assay has been adapted to three dimensions using melanoma spheroids, and inhibitors have been successfully evaluated in the three-dimensional assay. The evaluation of inhibitors in the assay has been submitted for publication. (See below.) The three-dimensional system closely mimics the environment that melanoma encounters during the metastatic (cancer spreading) stage. Third, the chemistry for producing the PICCO library has been fine-tuned. Some of the initial chemistry provided to be incompatible with the DNA coding tag. More facile chemistry conditions have since been employed to assemble the desired libraries. In particular, macrocyclic libraries containing peptide end groups are being prepared for screening against MT1-MMP and MMP-8.

Follow-on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Pahwa S, Bhowmick M, Amar S, Cao J, Strongin AY, Fridman R, Weiss SJ, Fields GB. Characterization and regulation of MT1-MMP cell surface-associated activity. *Chem Biol Drug Des.* 2019 Jun;93(6):1251-1264. doi: 10.1111/cbdd.13450. PMID: 30480376. PMCID: PMC6536371 [Available on 2020-06-01].

Palrasu M, Knapinska AM, Diez J, Smith L, LaVoi T, Giulianotti M, Houghten RA, Fields GB, Minond D. A novel probe for spliceosomal proteins that induces autophagy and death of melanoma cells reveals new targets for melanoma drug discovery. *Mol. Cancer.* Submitted 2019.

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

Organization: H. Lee Moffitt Cancer Center & Research Institute

Principal Investigator: Jennifer B. Permuth, PhD

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 this 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 14 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: 1) 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 2) 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.

So far, the research staff has 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. The researchers 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 have been conducted and recruitment has commenced one site at a time. This project 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. It will foster a valuable statewide 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.

Patents: None at the time of reporting.

Journals: None at the time of reporting.

3. Grant #8JK03: Chemoprevention of Lung Cancer in Former Smokers

Principal Investigator: Nagi B. Kumar, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Grant Progress Report: 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. Research staff, as well as others, have shown that curcumin (CUR) and omega-3 fatty acids (ω-3 FA) are effective at suppressing phosphorylated STAT3 (S*TAT3p)* and nuclear factor kappa-light-chain-enhancer of activated B cells (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 toward resolution of cigarette smoke-induced lung inflammation in former smokers. This research team, as well as 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 are administered independently. Based on this evidence, the research team hypothesizes that a standardized formulation of CUR plus 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. This will be mediated by reducing inflammation and through pro-resolving effects in the nodules. The team will test the hypothesis by using an experimental design and rigorously evaluating the safety and efficacy of a combination of ω-3 FA plus CUR or placebo administered for six months in former smokers, age 55 years or older, with lung nodules detected during the LDCT screening program. Results of the proposed trial may have immediate significant benefit to former smokers and other high-risk populations toward lung cancer prevention. Despite the reduced funding (cut by 50%), the goal is to obtain the safety and effectiveness of the combination of ω-3 FA plus CUR or placebo in 100 men and women who are diagnosed with the lung nodules.

In addition to full approval by the Moffitt Cancer Center Scientific Review Committee (SRC), researchers have now received full approval from the Institutional Review Board (IRB) to begin the trial. These approvals include protocol, informed consent form, questionnaires which are available by hard copy and electronic, study-specific brochures and flyers for patient recruitment. Amendment version No. 1.4 dated Oct. 15, 2018 was created and approved by the IRB and SRC. Amendment version No. 2 to expand the pool of subjects was created. All initial and amended regulatory approvals are complete. The study is active and recruiting subjects. Revisions have also been submitted to the FDA.

An initial investigators meeting has been completed. All delegation of authority and training logs have been signed by research staff. All investigators have been oriented to the full protocol. Re-training for modification/amendment No. 1.4 dated Oct. 15, 2018, was sent to all staff on the delegation of authority log. Re-training for modification/amendment version No. 2 was sent to all staff on the delegation of authority log after SRC and IRB approval.

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 an aggressive subtype of lung cancer that was selected by the National Cancer Institute for a focused strategic plan due to its high incidence in the United States, the lack of improvement on five-year survival rates, and as a direct response to the Recalcitrant Cancer Research Act (H.R.733) passed by Congress. SCLC is also the subtype of lung cancer most closely linked with tobacco exposure, which is an important focus of the James and Esther King Biomedical Research Program. Although SCLC is initially sensitive to standard chemotherapy and radiation therapy, tumor responses are short-lived. In addition, clinical investigational trials over the past four decades have been unable to significantly impact the cure rate of patients who present with advanced disease. The research team recently published new data showing that the immune system cannot normally recognize and infiltrate SCLC tumors and hypothesizes this may contribute to the modest benefit of current chemotherapy and immunotherapy treatments. The team also demonstrated that a modified myxomavirus (MYXV) can safely infect, induce a marked immune cell response and kill human SCLC tumor cells. In addition, research staff replicated these findings in a genetically engineered mouse model that replicates human SCLC genetics and biology. The researchers have now pursued design and implementation of strict methodology required for production of human clinical trial grade MYXV viral product for a “first-of-its kind” phase 1 clinical trial to test the safety of intratumoral delivery of MYXV to patients with advanced SCLC. The first year of this grant was dedicated to completing the needed regulatory and compliance paperwork to initiate this project. This included legal work completed with a signed Material Transfer Agreement between the University of Florida College of Medicine and the research team’s industry partner, DNAtrix. Research staff is proceeding with standard operating procedures for developing the Master Cell Banks for the GMP (Good Manufacturing Production) of MYXV and required safety testing in animals (as outlined in quarterly progress reports to the Department of Health’s James and Esther King Biomedical Research Program). The research team plans to meet in late 2019 with the FDA for a pre-investigational new drug (pre-IND) meeting to initiate the process for applying for an IND license and for approval to proceed with a phase 1 clinical trial that is scheduled for the last two years of this grant.

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 #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.
The purpose of this research project to determine the roles of hyaluronan (HA) metabolism in mechanisms of immune evasion and immune tolerance in bladder cancer. Obtained results will ultimately provide novel targets for bladder cancer therapy.

Several cancer types, including bladder, prostate, brain, lung and breast cancers are highly enriched with HA**.** HA accumulation in tumor tissues is 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. The research team's preliminary data strongly suggest an important role for tumor-derived HA and HA-mediated CD44 signaling in tumor-induced immunosuppression. These observations led the researchers 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 myeloid-derived suppressor cells (MDSC), promoting development of programmed death ligand 1-positive (PD-L1+) macrophages. To test their hypothesis, researchers have developed the following specific aims: Specific Aim 1 is to determine key molecular components involved in accumulation of immunosuppressive PD-L1-expressing myeloid cells in bladder cancer. Specific Aim 2 is to Investigate whether targeting the 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 a clinical study of patients with diagnosed bladder cancer as well as preclinical studies using an experimental animal model of bladder cancer. To date, researchers have collected clinical specimens (blood, tumor tissue) from 25 bladder cancer patients during surgery. Preliminary data indicate that metabolism of HA in bladder tumor tissue is severely affected and characterized by strong fragmentation and increased levels of HA fragments with low molecular weight. Such significant changes in HA metabolism support tumor growth through several mechanisms. Identification of these mechanisms and novel molecular targets is currently under way.

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 #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: Smoking during pregnancy is one of the major risk factors for spontaneous abortion, premature birth and low birth weight. Offspring of smokers also have an increased incidence of chronic behavioral problems, obesity, and nicotine addiction, starting during adolescence. Although many problems associated with prenatal nicotine exposure have been documented, understanding the mechanisms underlying them remains elusive. Emerging evidence suggests that a common factor behind the many problems may be an imbalance of the bacterial microbes in the gut or gut dysbiosis 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 prenatal nicotine exposure on the gut-microbiome-brain axis during two different time points: pregnancy and later when the offspring are adults. After the first year of the grant (2018-2019), the research group is close to completing the assessment of how chronic nicotine exposure alters the maternal gut microbiome and modulates metabolic byproducts released into the feces and circulation by bacteria in the gut during pregnancy. First, using an established rodent model, the data demonstrate that two weeks of exposure to nicotine in the non-pregnant state induces significant changes in the gut microbiome. This includes a significant increase in one group of bacteria (g_Clostridium) generally considered to be less beneficial. g_Clostridium decreases in the proportion of two other bacteria, one which is considered beneficial (g_Anaerostipes) and another one that is potentially detrimental (g_Prevotella). Parallel to changes in the bacterial balance in the gut in the non-pregnant state were significant changes in the types of metabolites, referred to as short-chain fatty acids, found in the feces and circulation, including isovaleric and hexanoic acid. These findings confirm that chronic nicotine exposure significantly impacts gut microbiome and provide a reference point for evaluation of the impact of nicotine exposure during pregnancy on the gut microbiome. In pregnant females, chronic nicotine exposure also significantly impacted the gut microbiome and fecal and circulating short-chain fatty acids, including an increase in the less beneficial gut bacteria, g_Clostridium, decreases in potentially beneficial short-chain fatty acids (butyric and propionic acid) and a general trend for nicotine to reverse pregnancy-induced upregulation of short-chain fatty acid levels. These findings support the original hypothesis and demonstrate that nicotine exposure in females, particularly during pregnancy, significantly impacts the gut microbiome and fetal exposure to certain short-chain fatty acids. Fortunately, the gut microbiome can be inexpensively re-balanced via changes in diet or the administration of antibiotic or probiotics. Thus, this 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 nicotine exposure in utero. Therefore, an important benefit of this research to the citizens of Florida is the potential to reduce the ongoing epidemic of obesity and cardiovascular disease in one-third of the population.

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: Ashok Saluja, PhD

Organization: University of Miami

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. This proposal examines the efficacy of Minnelide, a pro-drug synthesized in the lab

in targeting the tumor-stroma crosstalk and modulating the tumor microenvironment.

Present studies have accomplished the following:

The transforming growth factor-beta (*TGF-beta*)-mediated signaling was the major pathway in cancer-associated fibroblasts (CAFs) that was affected by Minnelide. To further analyze the pathways that are dysregulated by triptolide, the researchers conducted pathway enrichment analysis and found that pathways that are related to CAFs activation and functions, such as TGF-beta signaling, were down-regulated. As TGF-beta signaling is significant in CAFs phenotype, the research team visualized the deregulated genes in TGF-beta signaling pathway and showed that most deregulated genes lead to suppression of TGF-beta signaling. Furthermore, the downstream genes of TGF-beta signaling pathway were also down-regulated in the triptolide treated group.

The research team also has discovered that while Minnelide alone has a positive effect via the TGF-beta pathway, a combination therapy approach that involves Minnelide along with Gemcitabine, a typical standard of care medication used in treating pancreatic cancer, improves the survival rate in syngeneic mouse models. The researchers evaluated the efficacy of Minnelide with the standard of care combination of Gemcitabine with paclitaxel in syngeneic animal models of pancreatic cancer. The team also observed that in vivo, the combination study resulted in an extensive decrease in the stromal collagen. As mentioned previously, dense stroma is associated with poor outcomes in pancreatic cancer. Hence, the findings during this reporting period show progress in the stated aims.

Follow-on Funding: Florida Department of Health; *Evaluating Mechanisms of Stromal Modulation by Novel Anti-Cancer Drug Minnelide*; Ashok Saluja, PhD; $899,999.99;

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

8. Grant #8JK09: 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. The laboratory's recent work offers a potential solution. src homology 2 (SH2)-containing inositol phosphatase 1 (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. [The research team discovered that an anti-inflammatory compound, Apigenin (Api), enhances SHIP-1 expression in murine PC models. The researchers hypothesize that PC dampens SHIP-1 dependent signaling, causing increased MDSC and Treg activity, thus 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.

The findings for the following reporting period reflect the difficulties experienced when trying to reproduce tumor development data because there wasn't a thorough statistical analysis for a pending manuscript.

Panc02 cells were subcutaneously injected in SHIP knockout (KO) and SHIP wild type (WT) N2 C57BL/6 x 129Sv mice to create heterotopic tumor-bearing (TB) animal models. Tumor-bearing SHIP wild type (SHIP WT-TB) animals occurred over the course of 14 days after initially being subcutaneously injected with Panc02 cells. Even though the SHIP-KO-TB mice had a significantly higher tumor growth rate than the SHIP-WT-TB (as expected), the tumor measurements for the SHIP-KO TB mice didn't coincide with previous results from TB models.

Inconsistencies were also observed when collecting the tumor samples from the animal groups. The tumor sample for SHIP-KO-TB mouse 1 could not be located. This hindered our flow cytometry analysis of the tumor microenvironment (TME) for the SHIP-KO-TB and SHIP-WT-TB animals, which was needed for our pending manuscript. We were able to collect the spleen samples from all four animals and process them for flow cytometry experiments. G-MDSCs and M-MDSCs were evaluated between the two groups to show that SHIP-KO-TB mice had statistically higher percentages for both types of MDSCs relative to the SHIP-WT-TB mice. Additionally, the research team observed a considerable decrease in the effector CD8 T cell population and an increase in helper CD4 T cells for the SHIP-KO-TB mice. This could be due to the splenomegaly that was observed for the SHIP-KO-TB , which Is typically seen in cancer patients. These findings coincide with previous publications, showcasing that the lack of SHIP-1 expression causes an upregulation of MDSCs that promote tumor growth in PC animals. MDSC expansion in tumor-bearing animals suppress T cells (i.e. CD4, CD8) functionality. More specifically, CD8 T cells become deactivated due to decreases in Interferon gamma (IFNγ) production, which is a critical factor in eliminating cancer cells in the TME. Similar results were observed in the splenocytes of the mixed C57 black 6 (C57Bl/6) x 129Sv mice for the following study.

The research team also performed a cytometric bead array (CBA) analysis with the serum samples that were collected from each of the animal groups. This was done to evaluate the levels of various inflammatory cytokines between the SHIP-KO-TB and SHIP-WT-TB mice. Among the cytokines that were tested, interleukin (IL-6) and tumor necrosis factor (TNF) both displayed significantly high levels in the SHIP-KO-TB group compared to WT animals. Previous studies have established a relationship between cancer and chronic inflammation, indicating that cytokines influence the initiation, promotion, progression and metastasis stages of tumor development. IL-6 and TNF have been identified as protumor genic cytokines and are responsible for activating oncogenic transcription factors (i.e. NK-kB, AP-1, Signal transducer and activator of transcription 3 (STAT3) in many different types of cancers. Therefore, the findings Indicate that the rapid tumor growth rates that were observed in SHIP-KO-TB animals could be attributed to the high expression of cytokines, IL-6 and TNF, that triggered the cancer cell cycle.

Overall, the research team was able to obtain data from the C57BL/6 x 129Sv mixed animals that was relatively similar to results we achieved in the past. The only major problem was that the tumors didn't grow to the measurements that were needed in order to keep the team's findings consistent. Moving forward, the team plans to get SHIP KO mice that have been backcrossed onto a C57BL/6 background for at least 10 generations. To make this a possibility, the team will start its own SHIP KO and SHIP WT mouse colony. This will allow the team to have access to animals whenever needed for research projects. The findings will have a major Impact on the development of customized treatment techniques that can help reduce the rapidly Increasing death rates for PC.
Follow-on Funding: State of Florida; A Potential New Molecular Target for the Treatment of Pancreatic Cancer; Tamar Ghansah, PhD; $816,514.

Collaborations: The research team is currently collaborating with Jason B. Fleming, M.D. from Moffitt Cancer Center (Department Chair of GI Oncology) and Jose Trevino, M.D., from the University of Florida (College of Medicine/Department of Surgery for the purpose of gaining access to pancreatic cancer patient-derived xenograft (PDX) mice. PDX mice will prove to be beneficial for preclinical studies by providing more realistic and clinical outcomes for potential immunotherapy treatments for patients. Also, Dr. Trevino will be a co-PI on a NIH-NCI R01 grant.

Gerald Krystal, PhD (College of Medicine/Department of Pathology & Laboratory Medicine) and Laura Sly, PhD (College of Medicine/Department of Pediatrics and Vancouver), both from the University of British Columbia provided SHIP KO and SHIP WT mice for the most recent experiments.
Dr. Margaret Hibbs from Monash University (College of Medicine/Department of Immunology and Melbourne, Australia) to see if the team can obtain F10 SHIP HET breeding pairs with C57BL/6 background. Unfortunately, Dr. Kerr has yet to respond to our request for SHIP animals. We
The team is also working with Dr. Hibbs to receive F7 SHIP C57BL/6 animals from Australia. Dr. Hibbs will be supplying two females and two males SHIP -/+ for breeding purposes. (The team would essentially breed the animals to a F10 generation to get them as close to a C57BL/6 background as possible).

Jessica Luongo, an incoming freshman planning to major in biomedical sciences at the University of South Florida, will be trained by Krystal VIiiaiobos-Ayaia (lead research technician) to culture KPC 960 cells so that she can distinguish the immunoregulatory checkpoint receptors such as PD-1, which promotes tumor progression by deactivating CD8 T cells through the infiltration of PDL-1 into the tumor microenvironment. lmmunoregulatory checkpoint ligands and receptors will be detected In KPC cells treated with and without API via flow cytometry experiments. Villalobos-Ayala completed her master's in molecular medicine at USF on May 2. She has since transitioned into a full-time technician in the USF lab as of May 31. Luongo will also be trained in quantitative polymerase chain reaction (qPCR)/electrophoresis experiments.

The following students are also training in the USF lab:
Bradley Miller is a chemical engineering undergraduate student and a part-time tech in the lab. He helps with lab maintenance such as washing dishes, autoclaving, monitoring CO2 tanks and liquid N2 levels, biohazard disposal and specimen storage. His main project is developing a protocol for indirect immunofluorescence for various immune cells (i.e. lymphocytes, macrophages). Dr. Williamson will be working with him to develop the lab's immune cell staining protocols and histopathology staining of pancreatic tumors from our different PC pre-clinical models too.

Javier Areas is a graduate student in the master's program at USF. He is currently doing an internship in the USF lab where he has learned techniques involving both Western Blot and Image J to gain insight on the relationship between PC and SHIP-1 protein levels. His defined research project now is evaluating the role API has on SHIP-1 expression and its impact on the differentiation and polarization of macrophages (In vitro). He will now be learning and using flow cytometry, quantitative reverse transcription (qRT)-PCR, IDF, and Western Blots techniques for his define internship project in the lab.

Ari Marsh is a future medical student looking to gain research experience in cancer immunology. He will initially start working on basic lab techniques, eventually work up to tissue processing and data analysis. Marsh officially will start working in the lab on August 26, 2019.

Journals: None at the time of reporting

Patents: None at the time of reporting.

APPENDIX K

FISCAL YEAR 2018-2019 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
7JK01	University of Miami	Bramlett, Helen M.	$ 1,253,753	$ 848,501.00	$ 405,252.00	3/09/2017	2/29/2020	No	Yes	No
7JK02	H. Lee Moffitt Cancer Center	Chung, Christine	$ 1,896,200	$ 773,450.00	$ 1,122,750.00	3/16/2017	2/28/2022	No	No	No
7JK03	University of Miami	Dietrich, W. Dalton	$ 941,589	$ 627,726.00	$ 313,863.00	3/08/2017	2/29/2020	No	No	No
7JK04	H. Lee Moffitt Cancer Center	Gray, Jhanelle	$ 1,895,355	$ 758,142.00	$ 1,137,213.00	3/25/2017	2/28/2022	No	No	No
7JK05	University of Florida	Jiang, Zhihua	$ 1,422,150	$ 948,100.00	$ 474,050.00	3/07/2017	2/29/2020	No	Yes	Yes
7JK07	University of Florida	Fan, Z. Hugh	$ 125,000	$ 93,821.00	$ 31,179.00	6/15/2017	12/31/2018	No	Yes	No

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 research team performed experiments where reproductively senescent Sprague Dawley female rats were exposed to transient middle cerebral artery occlusion (tMCAO) and randomly assigned to either whole body vibration (WBV) or no-WBV groups. Animals placed in the WBV group underwent 30 days of WBV (40 Hz) treatment, performed twice daily for 15 minutes each session, five days each week. The motor functions of animals belonging to both groups were tested intermittently and at the end of treatment period. Brains were then harvested for inflammatory markers and histopathological analysis. The results demonstrate a significant reduction in inflammatory markers and infarct volume, with significant increases in brain-derived neurotrophic factor and improvement in functional activity after tMCAO in middle-aged female rats that were treated with WBV compared to the no-WBV group. These results were published in the paper listed below. The researchers have continued further analysis of inflammatory proteins and finished collection of brain tissue as well as blood for Western blotting and cytokine assays at 24 hours after tMCAO in both males and females, or 13 days after WBV/no WBV in females.

For the 24-hour analysis, researchers saw an increase in cytokine Interleukin (IL)-10 in female tMCAO rats exposed to nicotine compared to male tMCAO rats exposed to nicotine. For females at 13 days post-tMCAO, the team observed that post-stroke WBV reduces IL-2, IL-4 and IL-6 in the brain of nicotine-exposed female rats.

The researchers will continue to further analyze tissue and blood samples for other inflammatory markers. Based on previously reported data, the post-ischemic WBV intervention improves frailty parameters, reduces brain damage and reduces frailty in control female rats, but not in the nicotine-exposed group.

These data suggest that WBV may be a potential therapy to reduce post-ischemic frailty and improve functional and cognitive outcomes after stroke in women. Results of this study will define the frailty criterion that can be employed for future clinical studies.

Follow-on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Raval AP, Schatz M, Bhattacharya P, d'Adesky N, Rundek T, Dietrich WD, Bramlett HM. Whole body vibration therapy after ischemia reduces brain damage in reproductively senescent female rats. *Int J Mol Sci*.2018 vol 19 (9): 2749. PMID: 30217051. PMCID: PMC6164360.

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

Grant Progress Report: Aim 1 is to determine the Mesenchymal subtype signature and T-cell receptor clonality as predictive biomarkers of programmed cell death protein 1 (PD-1) inhibitors and determine the

tumor immune microenvironment (TIME) in the context of tobacco use in head and neck squamous cell carcinoma (HNSCC). Research staff identified 64 patients with detailed tobacco use history and formalin-fixed paraffin-embedded (FFPE) tumor blocks under the Institutional Review Board (IRB)-approved protocol (MCC 18754). The researchers will select the best 50 patients with sufficient and good quality tumors and perform NanoString PanCancer Immune Profiling and Progression Panels to define the Mesenchymal subtype.

The researchers are still locating the FFPE blocks because many of these blocks are located at the outside hospitals and sent to Moffitt Cancer Center. The enrollment of 45 patients for the proposed Phase I/II clinical trial of cetuximab and nivolumab has been completed. The demographic and toxicity data will be submitted for a presentation at the Society for Immunotherapy of Cancer. The clinical trial itself is supported by Lilly and Bristol-Myers Squibb. With the successful enrollment ahead of schedule by 11 months, both Lilly and Bristol-Myers Squibb allowed the team to add Cohort B, enrolling an additional 43 first-line recurrent/metastatic patients in the current protocol (now total sample size 88) and provided additional funding. This will ensure there are enough tissue specimens to complete the proposed experiments in Aim No. 1.

Aim No. 2 is to determine tobacco-specific genoproteomic changes that create immunosuppressive TIME in current smokers reflected by a lower Immunoscore compared to the never/former smokers. The researchers have completed development of the multiplex immunofluorescence (mIF) staining for the characterization of TIME using Perkin Elmer Vectra system and analysis pipeline using InForm and R package. Eighty of 80 proposed patients with FFPE tumor blocks have been identified and the detailed clinical data under the Institutional Review Board (IRB)-approved protocol (MCC 18754) has been collected. The mIF staining on these samples has been completed, and the researchers are analyzing the data to determine the Immunoscore. In addition, researchers analyzed 27 additional FFPE tumors matched with the frozen tumors that they performed whole-exome sequencing (WES) and RNA sequencing (RNAseq) on as described in Experiment 2. Data was analyzed to determine the Immunoscore and molecular signatures in context of their smoking status. In addition, DNA and RNA from 86 frozen HNSCC tumors have been isolated to date. The whole-exome sequencing from the 86 DNA samples and RNAseq from the 75 RNA samples by next generation sequencing are completed. The manuscript was submitted to a peer-reviewed journal and is currently under revision. The data will also be presented at the European Society of Medical Oncology Annual Meeting.

Aim No. 3 is to develop a smartphone-based assessment of patient-reported outcomes related to immunotherapy and smoking in HNSCC patients. The research team completed the selection of study measures and formatting for smartphone-based administration of patient-reported outcome measures related to immunotherapy and smoking in HNSCC patients. Seventy-two of 100 proposed patients have been enrolled to date.

Follow-on Funding: None at the time of reporting.

Collaborations: Ohio State University (James Rocco, MD, PhD) and Emory University (Nabil Saba, MD) are collaborators on the clinical trial described in Aim 1a. The clinical trial expenses for these sites are covered by Lilly Oncology and Bristol-Myers Squibb as sub-contracted sites under Moffitt Cancer Center. These sites will send the tissue samples from the clinical trial to Moffitt Cancer Center, which will be analyzed using the James and Esther King (JEK) grant. These collaborations will ensure the research team completes the patient accruals and tissue analyses and publishes the results within the JEK funding period.

M2Gen(Erin Siegel, PhD) is a collaborator which will allow us to analyze more samples for immunogenomic analyses in Aim 2. Due to the budget constraint of the JEK grant, research staff proposed a minimum number of samples to be analyzed to detect the effects of tobacco use. By allowing increased sample sizes, there is now enough statistical power to evaluate additional factors

as subset analyses beyond the effects of tobacco use in the immune system.

Journals: None at the time of reporting.

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: The researchers have focused on establishing and confirming a reproducible photothrombotic mouse stroke model, determining the injury's ability to spur natural neurogenesis in the dentate gyrus, and are beginning to assess the behavioral consequences of the lesion. The team has made great progress in establishing the model in the lab which produces a consistent lesion of the cerebral cortex in mice. The histological analysis of the initial mice was revealing in terms of the neuropathological changes. Therefore, the researchers' proposed use of Nestin-δ-thymidine kinase (TK) transgenic mice to produce a cortical infarct is feasible. The use of these transgenics will allow researchers to ablate the progenitor stem cell population and evaluate progenitor cell survival, cerebral atrophy and behavioral recovery. Researchers also optimized the behavioral assessment strategy for this study during the last year. There were some issues with the laser during the last year which included fixing the laser cooling system. This delayed the study somewhat in initiating the transgenic mouse studies. However, this problem has been fixed and the team has now produced cortical infarcts in wild-type mice and assessed the mice for behavior, which was reported in previous quarterly reports. The photothrombotic cortical infarct model is producing consistent behavioral deficits. Researchers then moved to producing the infarct in the transgenic mice, with and without ablation, using the Cytovene pumps followed by treatment with their proneurogenic compound. In the first attempts with the dose, it was discovered that the pumps were clogging and not releasing the Cytovene required to produce ablation. The correct dosage has now been determined through pilot studies. Researchers did have mice with control pumps in place and these animals have been assessed for behavior with the proneurogenic compound P7C3-A20, producing an improvement in sensorimotor and cognitive function. The histology for these animals is currently being assessed.

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 & Research Institute

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 a 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 2018 to June 2019, the clinical study has seen substantial progress in concurrence with its aims, goals and objectives. The first aim of this project is to establish the safety and early efficacy of 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. Subsequently, the study progressed to a Phase II trial commencing on May 29, 2019. Phase I trial results indicated a recommended safe dose of 150 mg of nintedanib adminstered by mouth daily (PO QD). Determining the MTD and DLT enhances the progression of this research into Aims 2 and 3 of the study which include correlative biomarkers of interest and resistance mechanisms in patients with NSCLC. Diligent efforts are being made to screen, consent and enroll patients in the Phase II trial portion of this study. Currently, the Phase II trial has 19 referrals, three consents, two enrollees and three participants screening which highlights dedicated efforts to achieve the goals of this project.

Culmination of these efforts has provided significant accomplishments based on study research. A “Phase I/II Study of Nivolumab and Ipilimumab Combined With Nentedanib in Advanced NSCLC” was published in the *Journal of Clinical Onocology* in June 2018. Phase 1 trial data was presented at the American Society of Clinical Oncology (ASCO) 2018 meeting in Chicago, and a recent abstract was approved for a poster presentation at the World Congress on Lung Cancer (WCLC) 2019 in Barcelona, Spain, highlighting the potential impact of this study on national and international cancer research. Data collected from this trial will serve as the foundation of a future biomarker-driven, randomized prospective trial. The serial blood and tissue collections are underway, and the assays for analysis are being optimized and validated internally by our team of experts.

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: None listed

Grant Progress Report: Aortic aneurysm is a life-threatening aortic disease. It is ranked among the top 15 of all causes of death in the United States. Epidemiological studies have revealed that the prevalence of aortic aneurysms ranges from 6% in men >65 years to 12.5% in men >75. What's even worse is that the prevalence is five to six times higher in tobacco users compared with the general population. Yet 16.1% of adult Floridians smoked as of 2017. However, mechanisms through which cigarette smoke exacerbates aortic aneurysm formation are currently poorly understood. This gap in knowledge stands as the most significant barrier in the field of research aimed at developing medical interventions to alleviate the risk of aortic aneurysm formation in cigarette smokers. The current project has three goals in the context of aortic aneurysm formation: 1) to develop an animal model capable of reliably recapitulating the exacerbating effect of cigarette smoke; 2) to evaluate how disruption of the immune function, which occurs frequently in smokers, exacerbates the pathogenesis and progression; and 3) to test the therapeutic potential of strategies aimed at correcting the disrupted immune function.

In the past year, the research staff of the project continued previous efforts in the model development. Through optimizing the chemical format and dosage of nicotine as well as integrating a “second hit” to

inhibit cross-linking of the matrix network, the group successfully created a mouse model that responds to nicotine treatment with a significant increase in aortic dilation. This model is characterized by early aortic tears and dissections, followed by progressive aortic medial degeneration. With this model, the researchers shifted the immune response in mice with developing aortic aneurysms with strategies such as adoptive transfer of type 1 T-helper (Th1) cells and type 2 T-helper cells (Th2), serological removal of interferon gamma (IFN), administration of recombinant IFN and genetic abrogation of Th1 differentiation. The results showed that interventions shifting the Th1/Th2 balance to the Th2 end accelerated aortic dilation and promoted aortic rupture, whereas administration of IFN to facilitate Th1 differentiation attenuated aortic dilation. Additionally, the group generated data suggesting that self-RNAs released by stressed and dying cells in aneurysmal aortas can activate toll-like receptor-7 (TLR7), resulting in production of inflammatory mediators. These observations led to a novel hypothesis that activation of TLR7 by self-RNAs constantly adds fuel to the inflamed aortic wall, substantiating the chronic inflammation and progressive aortic wall aneurysmal degeneration. The research staff submitted a multi-PI RO1 project to the National Institutes of Health (NIH) and successfully won an R01 grant to further elucidate the novel mechanism. A manuscript describing the critical role of an inflammatory mediator, named cyclophilin A, was submitted to the Federation of American Societies for Experimental Biology (FASEB) Journal and has been accepted for publication. These achievements have provided deep insights into the mechanisms underlying aortic aneurysm formation which may bring direct benefits to tobacco users down the road. Additionally, the new NIH funds will allow the group to expand this research program and hire more Floridians.

Follow-on Funding: NIH/National Heart, Lung, and Blood Institute (NHLBI); *Role of RNA-mediated Danger Signals in Regulating TAAD development*; Zhihua Jiang, PhD, Gilbert R. Upchurch, Jr., MD; $2,263,227 (pending).

Collaborations: None at the time of reporting

Journals: Zhou G, Liao M, Wang F, Qi X, Yang P, Berceli SA, Sharma AK, Upchurch GR Jr, Jiang Z. *Cyclophilin A contributes to aortopathy induced by postnatal loss of smooth muscle TGFBR1*. FASEB J. 2019 Oct;33(10):11396-11410. doi: 10.1096/fj.201900601RR. PMID: 31311317

Patents: None at the time of reporting.

6. Grant # 7JK07

Principal Investigator: Z. Hugh Fan, Ph.D

Organization: University of Florida

Grant Progress Report: We have designed several device layouts for various application scenarios. A simple design consists of a sample pad, flow channel, and detection zone. The sample pad is in diamond shape, with a side length of 2.5 mm. The flow channel is 1.6 mm wide and 10 mm long. The detection zone is a circle, with a diameter of 3.6 mm. Other device layouts are discussed in the related sections below.

The research team has fabricated paper-based devices by cutting and lamination, in a way similar to making an identification card. Our method employs a digital craft cutter to generate physical boundaries of paper according to the design of device, followed by a roll laminator to produce laminated devices. By encapsulating a paper strip between layers of thermally bonded polymer film, we have combined the simplicity of paper-based microfluidic device with the strength, durability, and flexibility of polymers to create low-cost, rugged microfluidic devices. Since the device design and fabrication are relatively straightforward, we have focused on developing enrichment methods. The enrichment step is critical for concentrating biomarkers in samples because it could determine if the assay is sensitive enough to detect exposure to second-hand smoke. One enrichment method is to take advantage of evaporation in a field environment or in a lab; this method is simple, low cost, and equipment-free. After waiting for a couple of minutes, the paper strip becomes dry due to evaporation in a normal laboratory environment. A small drop of water is applied to the bottom pad, and it sweeps the dye into the detection pad. The increase in the color intensity over the time is obvious, indicating a concentration process. Time in x-axis is shown in square because the flow distance is proportional to the square of time.

The device is made of polydimethylsiloxane (PDMS). A sample is dispensed into the device through a syringe from the center well, then spreads to all wells on the edge through the microchannels connecting the center well to the edge wells, all of which are filled with a red food dye for easy visualization. wells, and finally forms droplets above the edge wells. After minutes of evaporation, enrichment is achieved in all edge wells, and the concentrated sample is withdrawn back to the syringe.

The team studied the concentration effects of the devices with different designs. Devices with various number of wells or droplets have been fabricated and then investigated using a solution of the same volume. After evaporation in a controlled environment similar to the ambient condition, the concentration of the reagents collected was measured using LPAD.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: Valve-enabled Sample Preparation RNA Amplification in a Coffee Mug for Zika Virus Detection X. Jiang, J. C. Loeb, C. Manzanas, J. A. Lednicky, Z. H. Fan Angewandte Chemie International Edition Dec. 2018

Using Airbrushes to Pattern Reagents for Microarrays and Paper-fluidic Devices. Christopher Cassano, Teeodor L. Georgiev, and Z. Hugh Fan Microsystems Nanoengineering Dec. 2017

Patents: None at the time of reporting

APPENDIX L

FISCAL YEAR 2018-2019 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
6JK01	H. Lee Moffitt Cancer Center	Djeu, Julie Y.	$ 1,231,336	$ 1,162,928.40	$ 68,407.60	3/19/2016	8/31/2019	No	Yes	Yes
6JK02	H. Lee Moffitt Cancer Center	Drobes, David J.	$ 1,186,164	$ 728,487.80	$ 457,676.20	3/19/2016	2/28/2021	No	No	No
6JK03	University of Florida	Liao, Daiqing	$ 795,236	$ 744,325.30	$ 50,910.70	3/09/2016	8/31/2019	Yes	Yes	No
6JK04	Florida International University	Miguez, Maria Jose	$ 1,628,449	$ 983,525.22	$ 644,923.78	3/19/2016	2/28/2021	No	Yes	No
6JK06	H. Lee Moffitt Cancer Center	Park, Jong Y.	$ 1,231,336	$ 1,162,928.40	$ 68,407.60	3/21/2016	8/31/2019	No	No	Yes
6JK08	Florida Atlantic University	Wu, Jang-Yen	$ 1,231,336	$ 1,162,928.40	$ 68,407.60	3/31/2016	8/31/2019	Yes	Yes	Yes

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

Principal Investigator: Sheng Wei, MD

Organization: H. Lee Moffitt Cancer and Research Institute

Grant Progress Report: It is well-established that natural killer (NK)-mediated anti-tumor responses should be a key component of future immunotherapeutic applications. However, the study of NK cells in murine models has been hindered by the ability of NK cells to reach their intended target. In particular, the research team has demonstrated that NK cell infiltration into the tumor model depends on the stage of tumor growth and the metastatic (A549 stationary vs. H1299 metastatic tumor cell lines) immunosuppressive stage (higher tumor growth factor beta [TGF-beta] secretion in H1299) of the cancer cells.

In vitro, the researchers have developed a 3-D hydrogel model that aids in the analysis of cytokine production kinetics from tumor cells and their effect on trafficking NK cells. This model serves to elucidate further the role of cytokine-mediated distance communication between NK cells and tumor cells that leads to tumor evasion. These maneuvers are fully showcased in the inability of NK cells to not only survive in the immunocompromised NOD (non-obese diabetic) scid gamma (NSG) tumor-bearing mice but to traffick into the tumor, once established, allowing tumor growth. The most recent work demonstrates that enhancement of NK cells in tumor trafficking is mediated by the C-X3-C motif chemokine ligand 1 (CX3CL1). In this regard, this work contributes to the future study of NK cells in tumors. The research shows that the combined injection of IL-15, which aids in increasing the survival of NK cells, and the overexpression of CX3CL1 in tumor cells, which allows NK trafficking into the tumor, gives a comprehensive model to study intratumoral NK cells in preclinical applications. Moreover, the research staff has confirmed the restriction of CX3CL1 reduction as well as its receptor CX3CR1 in primary lung cancer experiments and in tissue slides, expanding the findings with The Cancer Genome Atlas (TCGA) databases to many solid malignancies increasing the potential impact of this work.

Researchers are still exploring the anti-miR183 or TGF-b anti-sense Manganese dioxide (MnO_2) nanoparticles that are part of the study. The team believes that the studies thus far are impactful to the community by 1) Providing more information about the obstacles posed to NK cells by the tumor; 2) Establishing an in vivo model to study future therapies aimed at improving NK cell function for therapeutic use; and 3) Allowing for the testing of the research team's own novel therapeutic delivery of payloads in nanoparticles to NK cells for function enhancement and overcoming tumor-mediated immunosuppression.

The research team believes that the funding provided for this study provides a clear basis for the previously referenced development both in lung cancer and beyond. It also will be the basis for several published reports and grant funding in the future, starting with the recent finalized funding of R21 for Dr. Sharma, several submitted publications from her lab, and an upcoming publication from the Wei lab on the immunosurveillance restriction by CX3CL1. The researchers expect that the investment of Floridians in this work will not only benefit cancer patients in the state but will also be the foundation for future work on NK cells that can lay the foundation for future therapeutic development.

Follow-on Funding: National Science Foundation; *Engineered Tumor Models to Study the Recruitment and Activation of Natural Killer Cells*; Blanka Sharma, PhD; $567,382.

Collaborations: None at the time of reporting.

Journals: None at the time of reporting.

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

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

Principal Investigator: Vani N. Simmons, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Grant Progress Report: This project is examining a novel smoking cessation intervention that utilizes reduced nicotine content in cigarettes prior to quitting, in conjunction 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.

During the current reporting period, research project staff finalized all study materials and obtained approval from the University of South Florida (USF) Institutional Review Board (IRB) to conduct the main randomized controlled trial (Study No. 2). Consequently, participant recruitment and data collection for Study No. 2 were initiated, with 33 participants randomized to date. Two hundred five were screened for eligibility via the telephone, and 189 completed study visits. Twenty-two treatment visits were completed, along with two six-month follow-ups.

In order to continue to optimize study procedures and generate a steady recruitment pace, the following protocol modifications were applied:

- Participants in the control condition receive supplementary handouts (to equate with written materials received in the targeted condition)
- Participants from the same street address are excluded in order to prevent contagion of information between participants
- Researchers adjusted the allowable time windows between treatment and follow-up visits, in order to permit greater scheduling flexibility
- The research team streamlined the telephone screening process by eliminating the Stages of Change algorithm as well as the Contemplation Ladder. Instead, motivation to quit smoking is now assessed by expressing interest to quit in the next 30 days
- The inclusion of a medical clearance letter for participants who may require outside medical approval in order to receive study product
- Participants may conduct follow-up assessments via the telephone if they are unable to attend these appointments in person

These modifications were reviewed and approved by the USF IRB.

As the research team is still recruiting participants for Study No. 2, sufficient data is not yet available to determine the actual impact to Floridians. Thus far, approximately 22% of study participants have reported continued smoking abstinence during their follow-up assessments. Nonetheless, cigarette smoking remains the top avoidable cause of death in Florida. The current project 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: Six undergraduate students from USF received training and worked on the project as research interns: Briana Merkher, Brooke Sprague, Camila Castro, Rachel Seng, Marco Cid and Maddison Clarke.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

3. 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: Breast cancer is the most commonly diagnosed cancer type and the second leading cause of cancer-related mortality for women. About one in eight women in the U.S. will develop invasive breast cancer over the course of her lifetime. In 2019, 268,600 new cases of breast cancer are expected to be diagnosed in women in the U.S., with 19,130 cases in Florida. Breast cancer has the highest incident rate and the second highest death rate among all cancer types with 115.5 incidents (2010-2014) and 19.8 deaths (2011-2015) per 100,000 in Florida.

Advanced breast cancer is still very difficult to treat and the prognosis for metastatic breast cancer remains poor. Therefore, development of new therapy for advanced breast cancer is urgently needed to improve treatment outcomes. Cyclic adenosine monophosphate (cAMP) response element binding (CREB)-protein and p300 (CBP/p300) are increasingly recognized as therapeutic targets for breast cancer. The goal of this grant is to understand the role of CBP/p300 in breast cancer biology and to test the effects of new pharmacologic agents targeting CBP/p300 for treating breast cancer in preclinical studies. New insights into mechanisms of action and effects of potent CBP/p300 inhibitors in breast cancer cells have been gained during the past year. Ultimately, the knowledge gained from this grant may lead to novel and effective therapies for treating advanced breast cancer.

Follow-on Funding: None at the time of reporting.

Collaborations: The following students have received training from Dr. Daiqing Liao's lab:

- Jessica Lewis, PhD candidate, Dept. of Anatomy and Cell Biology, University of Florida College of Medicine, Gainesville, Florida
- Iqbal Mahmud, PhD degree awarded, Dept. of Anatomy and Cell Biology, University of Florida College of Medicine, Gainesville, Florida
- Aaron Waddell, PhD candidate, Dept. of Anatomy and Cell Biology, University of Florida College of Medicine, Gainesville, Florida
- Jia Wang, PhD candidate, Beijing University of Chinese Medicine, Beijing, China

Journals: Mahmud I, Liao D. DAXX in cancer: phenomena, processes, mechanisms and regulation. *Nucleic Acids Res*. 2019 Sep 5;47(15):7734-7752. doi: 10.1093/nar/gkz634. PMID: 31350900. PMCID: PMC6735914.

Huo Z, Zhu L, Ma T, Liu H, Han S, Liao D, Zhao J, Tseng G. Two-Way Horizontal and Vertical Omics Integration for Disease Subtype Discovery. *Stat Biosci.* 2019 May 21:1-22. doi:10.1007/s12561-019-09242-6

Patents: *Polypeptide inhibitor of de novo lipogenesis in cancer cells*. Daiqing Liao, Iqbal Mahmud, Guimei Tian. University of Florida Research Foundation; International Patent Application. Patent Application Serial No. PCT/US2019/026011; filed 04/05/2019. Ref No.: UF#-17034 (222110-8200).

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

Principal Investigator: Maria Jose Miguez

Organization: Florida International University

Grant Progress Report: Although smoking was not seen as a main threat in the early days of the AIDS epidemic, currently, with the wide use of antiretroviral therapy, human immunodeficiency virus (HIV)-positive individuals are more likely to die from a tobacco-related disease than from HIV.

One of the national goals is to suppress viral load in all HIV-infected individuals. That cannot be easily achieved without a successful smoking cessation program, as smoking reduces the effectiveness of HIV treatment. Therefore, the public health burden of smoking is exponential.

This ongoing study is one of the largest tailored clinical trials designed to address this problem by providing tailored treatment to smokers living with HIV. The study is currently targeting 400 smokers and will reach up to 500 individuals, most of whom are minorities. Although it is premature to provide the effectiveness of this trial, data so far indicate that it is more successful than prior studies. Moreover, those who have not been able to quit have significantly reduced their smoking rates at least by half. Therefore, one can expect this trial to be highly cost-effective.

The scope of the burden of disease and death that cigarette smoking imposes on the public's health is extensive. However, the impact of smoking is particularly higher among women who prefer to use mentholated cigarettes. Therefore, assessing the effects of mentholated cigarettes was the major focus of analyses last year. Analyses indicated several adverse health effects of mentholated cigarettes beyond those caused by smoking non-mentholated ones. One of the most notable was the increase in weight observed among smokers of mentholated cigarettes. Therefore, the research team published two articles in open access journals with the aim to raise public awareness of the plausible weight effects of smoking mentholated cigarettes.

Follow-on Funding: None at the time of reporting.

Collaborations: The research team is actively collaborating with the University of Miami. Dr. Castro, a University of Miami faculty member, is the study physician of the clinical trial. The study procedures are administered at the Don Soffer Clinical Research Center, which is staffed by professionals with experience in clinical research involving people living with HIV. The research team is also performing laboratory testing at the University of Miami. The laboratory was selected because it participates in national external proficiency testing programs (National Institutes of Health, Centers for Disease Control and Prevention).

Journals: Míguez, MJ, Gray, CM, Castro J, Stanton CA., C Quiros, C, Bueno D., Kahler CW. Mentholated cigarettes or weight problems, which came first. *Adv Obes Weight Manag Control*. 2018;9(3):59-64.

Patents: None at the time of reporting.

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

Organization: H. Lee Moffitt Cancer Center and Research Institute

Principal Investigator: Jong Y. Park, PhD

Grant Progress Report: Prostate cancer disproportionally affects men of African Ancestry (AA) who have a much higher incidence and mortality rate than Caucasian men. In the state of Florida, approximately 1,700 AA cases are reported every year, according to the Florida Cancer Data System (FCDS) of the Florida Department of Health. Research staff proposed building a statewide biobank to support prostate cancer research among men of AA in Florida. The biobank has not been initiated due to various reasons, such as limited resources to establish the infrastructure for collaborative data and biospecimen collection.

The 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 from the National Institutes of Health (NIH), which will generate important scientific findings and ultimately lead to better strategies to reduce prostate cancer incidence and mortality.

As of June 30, 2019, the research team has received a total of 7,966 AA prostate cancer (PCa) cases during the ascertainment period from the Florida Cancer Data Registry. Among AA cases who sent information packets, while still waiting for response from 1,091 patients, a total of 240 were found to be deceased. To date, 1,071 have either consented or shown interest in participating, 1,399 declined either by mail (222) or phone (1,177), 1,222 could not be located, and 2,944 have not responded to the initial information packet/phone call after total of five attempts. This yields a participation rate of 15.9% (1091/6875). The adjusted participation rate, or the percentage of participants who consented to participate out of those who had communication with the research team, is 43.4% (1071/2469). Researchers observed that older AA PCa patients were found less likely to participate in the study.

The research team is also working on proposed epigenetic analysis. The researchers will analyze DNA methylation profiles between aggressive and indolent types with prostate tumor tissues. One hundred fifty tumor DNA samples have been extracted from AA cases Formalin Fixed Paraffin Embedded (FFPE) blocks for Aim No. 2 of the study.

Follow-on Funding: National Institutes of Health/National Cancer Instiute; *Research on Prostate Cancer in Men of African Ancestry: Defining the Roles of Genetics, Immunity and Stress Study (RESPOND)*; Chris Haiman (Univ. Southern California); $27,000,000.

Collaborations: University of Miami/Sylvester Cancer Center and the University of Florida Cancer Center at Jacksonville

Journals:

Patents: None at the time of reporting.

6. 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 progress made during this period includes several highlights.

First, the team was able to establish neuroprotection/neurogenesis of granulocyte colony-stimulating factor (GCSF) gene therapy in a bilateral carotid artery occlusion (BCAO) mouse stroke model. This was demonstrated by an increase of gamma aminobutyric acid (GABA) neurons after treatment with adeno-associated virus (AAV)- cytomegalovirus (CMV)-GCSF gene therapy. Gamma aminobutyric acid (GABA) neurons, identified as glutamic acid decarboxylase (GAD) positive neurons, increased by 50% in the AAV-CMV-GCSF gene therapy treated group compared to the group treated with the control vector, AAV-CMV-GFP. Similar results were obtained with the level of GAD65.

The research revealed an increase of neurons in the hippocampus as shown by Nissl staining**.**

There was a demonstration of neurogenesis in BCAO stroke model after AAV-GCSF gene therapy by immunofluorescence staining using specific antibodies to Nestin (green fluorescence), a marker for neural stem cells/ neural progenitors and Ki67 (red fluorescence), a biomarker of cell proliferation.

Furthermore, researchers noticed a demonstration of an increase in doublecortin, a biomarker for immature neurons in the AAV-CMV-GCSF mice.

Finally, the team detected a demonstration of the production of new immature neurons in the Dentate Gyrus in BCAO mice treated with GCSF gene therapy using double immunofluorescence of Bromodeoxyuridine (BrdU) and Doublecortin (DCX)

Follow-on Funding: Department of Defense; *Therapeutic intervention for non-motor symptoms in Parkinson's disease*; Jan-Weng Yu; $1,500,000 (pending).

Collaborations: None at the time of reporting.

Journals: Wu JY, Modi J, Menzie J, Chou HY, Tao R, Morrell A, Trujillo P, Medley K, Altamini A, Shen J, Prentice H. Granulocyte Colony Stimulating Factor (GCSF) Gene Therapy in Stroke and Alzheimer's Disease Model*. J Neurol Exp Neurosc.* 2018;4(1):S17.

Shu SY, Jiang G, Zheng Z, Ma L, Wang B, Zeng Q, Li H, Tan S, Liu B, Chan WY, Wu S, Zhu C, Li C, Wang P, Wu JY. A New Neural Pathway from the Ventral Striatum to the Nucleus Basalis of Meynert with Functional Implication to Learning and Memory. *Mol Neurobiol.* 2019 Oct;56(10):7222-7233. doi: 10.1007/s12035-019-1588-0. PMID: 31001802. PMCID: PMC6728281.

Patents: *Treatment for Ischemic Stroke*; Jang-Yen Wu and Howard Prentice; U.S. Patent Number: 10272063. Date issued: April 30, 2019.

Treatment for Ischemic Stroke; Jang-Yen Wu. International (PCT) Patent Application for (PCT/US2019/43451). Filed: July 25, 2019.

Granulocyte colony-stimulating factor (G-CSF) gene therapy for treating neurological diseases. Jang-Yen Wu. EFSID: 36063150. International Application Number: PCT/US19/33124. Filed: May 20, 2019.

APPENDIX M

FISCAL YEAR 2018-2019 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
5JK01	University of Miami	Lee, David	$ 1,953,000	$ 1,825,342.50	$ 127,657.50	5/25/2015	11/15/2019	No	Yes	No
5JK02	University of Miami	Campos, Michael	$ 1,951,531	$ 1,452,565.59	$ 498,965.41	5/25/2015	5/15/2020	No	Yes	Yes
5JK03	H. Lee Moffitt Cancer Center	Simmons, Vani N.	$ 1,904,351	$ 1,425,463.25	$ 478,887.75	5/25/2015	5/15/2020	No	Yes	No
5JK04	University of Florida	Kaye, Frederic J.	$ 1,414,858	$ 1,353,886.31	$ 60,971.69	5/25/2015	5/31/2019	No	Yes	Yes

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

Principal Investigator: David J. Lee, PhD

Organization: University of Miami

Grant Progress Report: Smoking tobacco is steadily related with mortality and morbidity, including health conditions such as heart disease, diabetes, respiratory illnesses and cancer. Prevalent health disparities associated with smoking exist among socioeconomic status groups and race/ethnicities. This study seeks to eliminate this disparity in three racial/ethnic groups using an intervention with promising preliminary findings. The proposed study will be the first randomized controlled trial (RCT) to test the efficacy of cognitive behavioral therapy (CBT) to eliminate racial/ethnic differences in key psychosocial factors that influence smoking behavior and to eliminate cessation disparities. This research also will be the first to explore the relationship between hypothalamic pituitary adrenal (HPA) axis functioning, which is one's central stress response system, and race/ethnicity among treatment-seeking smokers. Cortisol, the stress hormone, is attenuated in response to stress among smokers, the slope of which predicts relapse. Also, both persistent elevations and declines of cortisol in response to stressors are indicators of general poor health and overuse of the HPA axis. Less is known about the links between naturally occurring stressors (e.g., quitting smoking and nicotine withdrawal), depressive symptoms and cortisol. In addition, this study has strong potential to move the field forward and shift current research and clinical practice in a way that most cessation trials do not. It will address an important public health problem: understanding and reducing tobacco-associated health disparities. It is known that a significant proportion of health disparities could be eliminated if smoking was meaningfully addressed. This project will set the stage for larger scale studies to elucidate these relationships and improve the health of smokers.

In this period, 60 individuals were screened, 47 of which were eligible and 16 were enrolled in the study. A total of six group cohorts were initiated during this period.

Over the last period, the research team revised the recruitment strategies to recruit and retain approximately equal proportions across the three demographics included in the trial. At the University of Miami (UM) site, the recruitment strategies mainly targeted Hispanic participants since the study already had reached its goal for African Americans, and the Moffitt Cancer Center site focused its efforts on the recruitment of non-Hispanic whites. To increase the number of Hispanic participants, all the study materials were translated and delivered to Spanish speakers. This tactic improved recruitment efforts significantly; however, it had an impact on the study timeline. A no-cost extension was requested in order to complete all the follow-up assessments with the remaining group cohorts. Moffitt Cancer Center has now completed all the 12-month follow-up assessments. The study team continued monitoring each of the study milestones, data quality and retention efforts. Preliminary data are still being analyzed to study racial/ethnic differences in perceived discrimination and relationship to overall smoking quit attempts. The scientific direction of the study remained the same.

Follow-on Funding: None at the time of the reporting.

Collaborations: In the Moffitt Cancer site: Amanda Palmer, a graduate student in the Department of Psychology at the University of South Florida (USF), Tampa; Ifrah Rizwan and Mikaela Hemenway, two USF psychology undergraduate students; and Ursula Martinez, PhD, a Moffitt Cancer Center postdoctoral fellow, received training and performed research under this project.

At the University of Miami site, there was one Master of Public Health (MPH) student, Asmith Joseph, from Florida International University, performing research activities under this project.

Journals: Webb Hooper M, Lee DJ, Simmons VN, Brandon KO, Antoni MH, Unrod M, Asfar T, Correa JB, Koru-Sengul T, Brandon TH. Reducing racial/ethnic tobacco cessation disparities via cognitive behavioral therapy: Design of a dualsite randomized controlled trial. *Contemp Clin Trials* 2018 May;68:127-132. doi: 10.1016/j.cct.2018.03.017. PMID: 29617633.

Webb Hooper M, Asfar T, Unrod M, Dorsey A, Correa JB, Brandon KO, Simmons VN, Antoni MA, Koru-Sengul T, Lee DJ, Brandon TH. Reasons for exclusion from a smoking cessation trial: an analysis by race/ethnicity. *Ethn Dis* 2019 Jan 17;29(1):23–30. doi:10.18865/ed.29.1.23. PMID: 30713413. PMCID: PMC6343546.

Patents: None at the time of the reporting.

2. 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 first goal of this work is to study if electronic cigarette (EC) vapors are toxic to cells and what they may be doing to the normal lining of one's airways. For this research, cells from the nose and cells from airways are studied and grown in the laboratory in small dishes called plates.

One important finding is that vaping e-liquid without nicotine, for 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 exists over the cells, important for lung protection (the so-called mucociliary clearance system).

In normal volunteers, the same vaping effects were observed when measuring cells from the nose. If the e-liquid consists of pure vegetable glycerol (a common e-liquid), the effect seems to be worse.

Another important finding is that adding nicotine content in e-cigarettes disrupts mucociliary clearance, making the essential defense mechanism of the lung even worse. It was discovered in one of the molecular mechanisms how nicotine does this and how it can be blocked with a specific substance.

On a parallel trial under this same grant, the effects of switching tobacco smokers to EC was studied. This is hard to do as only one in five smokers can do this and keep on exclusive EC for three months. This part of the study is still ongoing, and enrollment is running as expected. So far, it has been learned that subjects who can do the switch to EC are the ones who inhale the longest, and new participants are being taught how to do so. It has been learned that doing the

switch from cigarettes to EC significantly changes the bacteria that live in the mouth. The effects of these bacterial changes are being analyzed.

Follow-on Funding: National Institutes of Health; *TRP-Mediated Airway Inflammation by E-cigarette Vaping*; Matthias A. Salathe*,* MD; $1,941,197.

Collaborations: Dr. Santanu Banerjee (University of Miami) to study oral microbiome changes associated with changing tobacco smoking to EC vaping; Drs. Robert Foronjy and Patrick Geraghty, State University of New York (SUNY) Downstate, on signaling with tobacco smoke and e-cigarette vaping; Mass Spectrometry Laboratory at the University of Kansas Medical Center (KUMC) measuring nicotine levels; Dr. Marianne Geiser, Institute of Anatomy, University of Bern, Switzerland, on e-cigarette vaping; Dr. Nikki Nollen, Department of Preventive Health, KUMC, on menthol JUUL vaping; and Dr. Sunil Abhyankar, Stem Cell Institute at KUMC, on using mesenchymal stem cells on e-cigarette vaping and tobacco smoking.

Journals: Nath S, Ohlmeyer M, Salathe MA, Poon J, Baumlin N, Foronjy RF, Geraghty P. Chronic cigarette smoke exposure subdues PP2A activity by enhancing expression of the oncogene CIP2A. *Am J Respir Cell Mol Biol* 2018 Dec;59:695–705. PMID: 30011381.

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* Published Online: June 7, 2019. doi: 10.1164/rccm.201811-2087OC. PMID: 31170808.

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;200:51-62. doi: 10.1164/rccm.201808-1518OC. PMID: 30641028. PMCID: PMC6603057 [Available on 2020-01-01]

Patents: None at the time of the reporting.

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

Principal Investigator: Vani Nath Simmons, PhD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Grant Progress Report: Tobacco smoking is the leading preventable cause of cancer mortality. Counseling, although effective in aiding smoking cessation, is rarely chosen by smokers. In contrast, minimal self-help interventions, such as smoking cessation booklets have much wider potential reach, yet their efficacy has been largely disappointing, with incremental abstinence rates averaging only 1% except for the extended self-help smoking interventions developed by the Moffitt Cancer Center research team. These cessation-focused English-language booklets, Stop Smoking for Good, distributed to smokers over the course of 18 months, were found to be cost-effective and efficacious. Given the high dissemination potential, any significant improvement in the efficacy of self-help would have a large public health impact on smoking and smoking-related illness and mortality. Availability of a validated Spanish-language version would enhance the intervention’s public health impact by reaching the largest

and fastest growing ethnic minority population of smokers. In Florida, smoking prevalence among Hispanics is greater than the national prevalence. Hispanic smokers face unique challenges such as lower awareness and acceptance of pharmacotherapies and less cessation assistance from health providers.

The goal of this study is to expand the reach of evidence-based, self-help interventions 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.

The first aim is to develop a culturally appropriate self-help intervention for diverse 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.

The second aim is to test the efficacy of the self-help intervention among Spanish-speaking smokers. 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 (NCI). The hypothesis is that SS-SP will produce higher abstinence rates than UC through 24 months.

In prior reporting periods, the first aim was completed resulting in the development of a Spanish-language smoking cessation intervention: a series of 10 booklets, nine supportive pamphlets, and a family support booklet that addressed unique barriers and issues relevant to Hispanic smokers. The randomized controlled trial (RCT) testing the effectiveness of the newly created Spanish-language intervention compared to the NCI booklet (the second aim) is ongoing. Assessments are conducted every six months for two years.

During the previous reporting period, recruitment for the RCT was completed, with 881 Hispanic smokers screened and 555 participants who were found eligible, enrolled in the study, and returned baseline assessments. During the current reporting period, administration of the six-month and 12-month follow up assessments, as well as the biochemical verification of smoking abstinence at the 12-month time point, were completed. Administration of 18-month and 24-month follow-up assessments and biochemical verification of smoking abstinance at the 24-month time point have begun and are ongoing. The completed baseline, six-month and 12-month follow-up data are being coded and prepared for analyses. A paper describing the creation of the self-help materials was published and another manuscript describing the design, methods, analysis plan and baseline characteristics of the ongoing RCT was submitted for publication.

Follow-on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Piñeiro B, Díaz DR, Monsalve LM, Martínez Ú, Meade CD, Meltzer LR, Brandon KO, Unrod M, Brandon TH, Simmons VN. Systematic transcreation of self-help smoking cessation materials for Hispanic/Latino smokers: improving cultural relevance and acceptability. *J Health Commun*. 2018;23(4):350–359. doi:10.1080/10810730.2018.1448487. PMID: 29533167. PMCID: PMC5972386.

Patents: None at the time of reporting.

4. Grant #5JK04: First-of-its-Kind Study of Oncolytic Virotherapy for Small Cell Lung Cancer (SCLC) Using Mouse Models and Human Ex-Vivo Intralesional Analyses

Principal Investigator: Frederic Kaye, MD

Organization: University of Florida

Grant Progress Report: Small cell lung cancer (SCLC) is an aggressive subtype of lung cancer that was selected by the National Cancer Institute for a focused strategic plan due to the high incidence in the United States, the lack of improvement on five-year survival rates, and as a direct response to the Recalcitrant Cancer Research Act (H.R.733) passed by Congress. SCLC is also the subtype of lung cancer most tightly linked with tobacco exposure that represents an important focus for the James and Esther King Grant Program. Although SCLC is initially sensitive to standard chemotherapy and radiation therapy, tumor responses are short-lived. In addition, clinical investigational trials over the past four decades have been unable to significantly impact the cure rate of patients who present with advanced disease.

The research team recently published important new data showing that the immune system cannot normally recognize and infiltrate SCLC tumors, and it came to hypothesize this may contribute to the inability to cure advanced SCLC using current chemotherapy and immunotherapy treatments. It also demonstrated that a modified myxomavirus (MYXV) can safely infect, induce a marked immune cell response and kill human SCLC tumor cells. In addition, the team replicated these findings in two different genetically engineered mouse models that replicate human SCLC genetics and biology.

Over the past year, researchers have performed additional experiments to extend these pre-clinical findings. This new data was included in the revised version of the manuscript published in a high-impact scientific journal. This published report has further strengthened the rationale supporting a future research clinical trial to test the safety and efficacy of intratumoral delivery of MYXV in patients with advanced SCLC.

For example, the team independently confirmed and rigorously quantitated the ability of MYXV to efficiently infect and inhibit tumor growth in human and mouse SCLC tumor models. The team also studied the mechanism of MYXV-mediated SCLC tumor cell killing and examined the ability of MYXV to induce sustained immune cell infiltration within SCLC tumor masses. In addition, the team continued experiments testing the strategy of combining intratumoral delivery of MYXV with concurrent standard chemotherapy and anti-PD1/anti-CTLA4 immunotherapy. These experiments over the past year have also provided preliminary data on the safety of delivery of MYXV in animal models.

The team plans to use this preclinical data obtained in the past year to design and implement a new “first-of-its kind” phase one clinical trial to study the safety and efficacy of intratumoral delivery of MYXV to patients with advanced SCLC.

Follow-on Funding: Florida Department of Health (James and Esther King Biomedical Research Program); *Good Manufacturing Practice (GMP) production to allow phase one clinical trial testing intralesional delivery of myxomavirus to patients with advanced small cell lung cancer*; Frederic Kaye, MD; $1,360,857,

Collaborations: This project is a collaboration between the Departments of Medicine, Molecular Genetics & Microbiology, and Anatomy and Cell Biology within the University of Florida (UF) College of Medicine. Daniel Shabashvilli is a postdoctoral fellow working on this project. Patrick Kellish is a UF Biomedical Sciences graduate student who is working on human patient-derived xenografts (PDX) models for small cell lung cancer and also on immunocompetent allograft mouse models to optimize immunotherapy activation with oncolytic virotherapy for lung cancer.

UF undergraduate students are also working on this project. Connor Hertzell, an undergraduate student, received a Research Scholar award from UF to work on this project.

The research team has collaborated with the Tumor Immunology Program at Moffitt Cancer Center for conducting immunotherapy trials in lung cancer. It hopes to partner with DNAtrx in Houston and the University of Florida GMP manufacturing facility to generate MYXV for human phase one clinical trial.

Journals: Kellish P, Shabashvili D, Rahman MM, Nawab A, Guijarro MV, Zhang M, Cao C, Moussatche N, Boyle T, Antonia S, Reinhard M, Hartzell C, Jantz M, Mehta HJ, McFadden G, Kaye FJ, Zajac-Kaye M. Oncolytic virotherapy for small-cell lung cancer induces immune infiltration and prolongs survival. *J Clin Invest* 2019 Apr 29;129(6):2279-2292. doi: 10.1172/JCI121323. PMID: 31033480. PMCID: PMC6546459.

Patents: None at the time of reporting.

APPENDIX N

FISCAL YEAR 2018-2019 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
8JK08	University of Miami	Merchant, Nipun	$ 99,999	$ 60,309.70	$ 39,689.30	4/25/2018	9/30/2018	No	No	Yes

Grant # 8JK08

Principal Investigator: Nipun Merchant, Ph.D.

Organization: University of Miami

Grant Progress Report: Major contributors to therapeutic resistance in pancreatic cancer (PDAC) include Kras mutations, a dense desmoplastic stroma that prevents drug delivery to the tumor, and activation of redundant signaling pathways. We have previously identified a mechanistic rationale for targeting STAT3 signaling to overcome therapeutic resistance in PDAC. We have now investigated the molecular mechanisms underlying the heterogeneous response to STAT3 and RAS pathway inhibition in PDAC. Effects of JAK/STAT3 inhibition (STAT3i) or MEK inhibition (MEKi) were established in *Ptf1a$^{cre/t}$;LSL-Kras$^{G12D/+}$;* Tgfbr2$^{flox/flox}$ (PKT) mice and patient-derived xenografts (DDX). Amphiregulin(AREG) levels were determined in serum from human PDAC patients, LSL—*Kras$^{G12D/+}$;Trp53$^{R172H/+}$;Pdx1$^{Cre/+}$* (KPC), and PKT mice. MEKi/STAT3i-treated tumors were analyzed for integrity of the pancreas and the presence of cancer stem cells (CSC). We observed an inverse correlation between ERK and STAT3 phosphorylation. MEKi resulted in immediate activation of STAT3 , while STAT3i resulted in TACE-induced, AREG-dependent activation of EGRFR and ERK. Combined MEKi/STAT3i sustained blockade of ERK, EGFR, and STAT3 signaling, overcoming resistance to individual MEKi or STAT3i. This combined inhibition attenuated tumor growth in PDX and increased survival of PKT mice while reducing serum AREG levels. Furthermore, MEKi/Stat3i altered the PDAC tumor microenvironment by depleting tumor fibrosis, maintaining pancreatic integrity, and downregulating CD44+ and CD133+ CSC. These results demonstrate that resistance to MEKi is mediated through activation of STAT3, while TACE-AREG-EGFR-dependent activation of RAS pathway signaling confers resistance to STAT3 inhibition. Combined MEKi/STAT3i overcomes these resistances and provides a novel therapeutic strategy to target the RAS and STAT3 pathway in PDAC.

We have now also identified a novel mechanism showing that combined MEKi and STAT3i also inhibits tumor fibrosis and enhances $CD8^+$ cytotoxic T-cell (CTL) infiltration to the tumor while downregulating immunosuppressive regulatory T cells (T_{regs}) and myeloid derived suppressor cells (MDSCs) in the TME, resulting in reduced tumor burden and improved survival in genetically engineered mouse models (GEMs) of PDAC. In addition, we show that the tumor suppressive effects of MEKi and STAT3i are T cell dependent. This change in the TME, however, is accompanied by sustained PD-L1/PD-1 and CTLA-4 expression. Our results further show that combined MEKi and Stat3i with PD-1 inhibition can harness the effects of immune checkpoints inhibitors for an enhanced anti-tumor response. Based on this data, we are moving forward to start a clinical trial of MEKi/STAT3i and PD-1 inhibition in patients with advanced pancreas cancer.

Follow-on Funding: None at the time of reporting

Collaborations: None at the time of reporting

Journals: Nagathihalli NS, Castellanos J, Lamichhane P, Messaggio F, Shi C, Dai X, Rai P, Chen X, VanSaun M, Merchant NB. Inverse Correlation of STAT3 and MEK Signaling Mediates Resistance to RAS Pathway Inhibition in Pancreatic Cancer. Cancer Research, 07/2018

Patents: None at the time of reporting

Live Like Bella Pediatric Cancer Research Initiative

APPENDIX O

FISCAL YEAR 2018-2019 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
9LA01	Florida State University	Sang, Q.X. Amy	$ 250,000	$ 0.00	$ 250,000.00	4/17/2019	3/31/2022	No	No	No
9LA02	H. Lee Moffitt Cancer Center	Druta, Mihaela	$ 784,733	$ 0.00	$ 784,733.00	3/29/2019	3/31/2023	No	No	No
9LA03	H. Lee Moffitt Cancer Center	Smalley, Keiran	$ 250,000	$ 0.00	$ 250,000.00	4/02/2019	3/31/2022	No	No	No
9LA04	University of Florida	Lamba, Jatinder	$ 223,758	$ 0.00	$ 223,758.00	4/05/2019	3/31/2022	No	No	No
9LA05	University of Florida	Qian, Zhijian	$ 250,000	$ 0.00	$ 250,000.00	4/11/2019	3/31/2022	No	No	No
9LA06	University of Miami	Rodrigues, Claudia	$ 250,000	$ 0.00	$ 250,000.00	4/03/2019	3/31/2022	No	No	No
9LA07	University of Miami	Robbins, David	$ 250,000	$ 0.00	$ 250,000.00	4/29/2019	4/30/2022	No	No	No
9LA08	University of Miami	Marples, Brian	$ 250,000	$ 0.00	$ 250,000.00	4/01/2019	3/31/2022	No	No	No
9LA09	University of Miami	Trucco, Matteo	$ 241,509	$ 0.00	$ 241,509.00	4/08/2019	3/31/2022	No	No	No
9LA10	University of South Florida	Acevedo-Duncan, Mildred	$ 250,000	$ 0.00	$ 250,000.00	5/21/2019	4/30/2022	No	No	No

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

Principal Investigator: Qing-Xian Amy Sang, PhD

Organization: Florida State University

Grant Progress Report: Brain and other central nervous system cancers are one of the most common types of cancer in children. Atypical teratoid rhabdoid tumor (ATRT) of the brain is the most lethal type of human pediatric brain cancer. This proposed project is building a novel 3-D spheroid model that mimics human pediatric brain rhabdoid tumor formation. The state-of-the-art clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (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 objective is to generate a novel human induced pluripotent stem cell (HiPSC)-derived ATRT organoid model for drug testing. 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 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1) tumor suppressor. Thus, deleting the SMARCB1 gene in early NPCs may generate a rhabdoid tumor model for therapeutic evaluation. ATRT is a rare and very aggressive type of human pediatric brain cancer that mostly arises from the cerebellum located at the hindbrain region. Thus, a human cerebellum brain organoid model will be built using Episomal iPSC. 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 knockout construct was transfected into induced pluripotent stem cells, and experiments will be performed to verify if the SMARCB1 gene is knocked out. DNA sequencing experiments will be carried out to verify the gene knockout, and western blot experiments will further verify the SMARCB1 protein is not produced by the stem cells.

Treatments for ATRT are ineffective due to the lack of understanding of the molecular mechanisms and effective preclinical investigative models for drug testing. The treated patients have a high recurrent rate with a 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 in a child and an adult. Thus, this research may lead to a novel and powerful experimental ATRT organoid model to be used for drug screening, evaluation, and testing. The impacted population is young children. Malignant rhabdoid tumor of the brain is the most lethal type of human pediatric brain cancer, responsible for half of all pediatric brain cancer deaths. ATRT is a rare and very aggressive cancer that mainly arises from the children’s and infant’s cerebellum. Effective treatments for ATRT are medically unmet due to the lack of a compelling human ATRT model for drug testing and therapeutic evaluation. The research team is constructing a human organoid model that mimics human pediatric malignant rhabdoid tumors to identify highly efficacious therapies for human pediatric cancer patients. This work will also advance the knowledge of how the loss of inactivating SMARCB1 can prone cells to become cancerous.

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 Azacytidine in Patients With Recurrent, Resectable Osteosarcoma

Principal Investigator: Mihaela Druta, MD

Organization: H. Lee Moffitt Cancer Center and Research Institute

Grant Progress Report: Osteosarcoma is the most common bone cancer in pediatric and young adult patients. Unfortunately, cure rates have not improved over the past three decades despite many efforts. Osteosarcoma is particularly difficult to cure if it recurs. In osteosarcoma genetic material, the DNA has become scrambled with tens of thousands of breaks. This is thousands of times more frequent than in most other childhood cancers and has made figuring out which of these changes matter elusive. To date, there are not clear genes that can be targeted with chemotherapy in a smarter way for osteosarcoma.

There is a potential opportunity that all these complex genetic changes may offer: immunotherapy. There have been decades of observations that some osteosarcomas can stimulate the patient's immune system, and the immune system can shrink or control osteosarcoma. Several trials have sought to prove that agents that stimulate the immune system could be efficacious in osteosarcoma, but thus far, none have demonstrated success.

The planned study builds on rapidly developing discoveries in the field of immune oncology: training the immune system to fight cancer. This field works to understand tumor cells, immune cells and the environment in which these cells interact—many complicated interactions. Researchers typically focus on one aspect of this interaction. There is evidence that combining two agents increased the chances that the immune system would recognize and destroy osteosarcoma cells.

The trial builds from this evidence and from observations in other genetically complex cancers to combine Azacytidine and Nivolumab in osteosarcoma patients that have had their disease recur. While it is often difficult to obtain access to exciting agents in pediatric cancers, researchers have been able to obtain access to this agent through ongoing work with Bristol-Myers Squibb, which is committed to supplying Nivolumab for this trial. Researchers will give these compounds to patients who have had osteosarcoma and seen it recur. The patients will then proceed to surgery to remove all the tumors. Both agents will be given after surgery as well to determine if they can keep osteosarcoma from coming back again. The nearly 40 patients that will be treated on this trial would be expected to have another recurrence 80% of the time without this therapy. The research team is hypothesizing that this treatment can more than double chances of osteosarcoma not coming back within a year from surgery.

The trial will be conducted through the National Pediatric Cancer Foundation's Sunshine Project. This network was born in Florida and is coordinated through Moffitt Cancer Center which is also the location of the principal investigator. The budget covers the Florida costs of this trial including the coordinating center's major part in conducting this trial safely. There are currently six active Florida sites that conduct trials through this mechanism; an additional 12 sites around the nation would participate in this trial. These trials are available to all Florida

patients and to patients across the country. The Sunshine Project has a successful track record of completed trials in pediatric cancers in Florida and beyond.

During the reporting period of March 29-June 30, 2019, the research project was still in the regulatory process of opening to accrual. Therefore, there is no scientific progress to report for this time period.

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: H. Lee Moffitt Cancer Center and Research Institute

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 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 this proposal, the research staff will use genetically engineered mouse models to determine the link between the age of burning UVR exposure and melanoma development. Researchers will then perform a comprehensive genomic analysis of pediatric melanoma specimens and use the information to build improved genetic mouse models of pediatric melanoma.

Improving understanding of pediatric melanoma biology and the impact of UVR during childhood on subsequent melanoma development is vitally important to inform prevention campaigns, including increasing sun awareness and skin protection, and implementing age-appropriate tanning bed restrictions. It also will allow the development of treatment strategies that are truly specific to pediatric melanoma patients rather than those adopted from their adult counterparts.

Follow-on Funding: None at the time of reporting.

Collaborations: This project is a collaboration between five investigators at the Moffitt Cancer Center. 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 Hematopoietic Stem Cell Transplantation (HSCT) Recipients

Principal Investigator: Jatinder K Lamba, PhD

Organization: University of Florida

Grant Progress Report: Since the award of this grant, the three main investigators have held regular in-person and video conference meetings in order to discuss implementation of the experimental plan, data acquisition and patient recruitment. The research staff has also secured the regulatory documents (e.g., IRB submissions, material data agreements) necessary to start the laboratory component of this work. Researchers have identified different cohorts of patients treated with chemotherapeutic agents in question and are processing the DNA samples for pharmacogenomics analyses. These analyses will provide additional pharmacokinetic and pharmacogenomics data necessary for development of mathematical models which will be used for data analysis from the main research cohort.

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: The researchers have submitted modifications to the current animal protocol to include new animal work which was proposed in their research proposal. The revised protocol has been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida on June 20, 2019, and the research project has been initiated.

The team worked to isolate a cluster of differentiation 34 (CD34+) cells from human cord blood. To determine whether ecotropic virus integration site 1 protein homolog (EVI1) affects the expression of Neurogenin-1 (NGN1) in human cord blood CD34+ cells, the researchers first obtained human umbilical cord blood (UCB) from the New York Blood Center. Mononuclear cell (MNC) factions were isolated from UCB by Ficoll-Paque premium density gradient. CD34+ cells were enriched from MNCs through positive immunomagnetic selection. The purity (90-95%) of the isolated CD34+ cells was confirmed by flow cytometric analysis using CD34-PE-cy7 and CD34-APC antibodies.

Researchers have also optimized the culture conditions for expanding CD34+ cells in vitro to obtain sufficient samples for these studies. The stem-span II medium containing cytokines, including human stem cell factor (SCF), thyroid peroxidase (TPO), fibroblast growth factor (FGF1) and heparin, was selected to be used for CD34+ cell culture. At nine days, the culture of CD34+ cells was expanded nine-fold in vitro.

The research team has subcloned EVI1 into SFLV lentiviral and protocadherin (PCDH) lentiviral vectors. The control vector and the vector expressing EVI1 were expressed in 293 T cells together with lentiviral packaging plasmids to generate lentivirus. Researchers first infected human leukemia cell line U937 cells with virus expressing EVI1 and control vector. The expression of EVI1 in U937 cells using both lentiviral vectors was determined by western blot. The team discovered that both lentiviral vectors can express EVI1 in human U937 cells. Currently, researchers are infecting CD34+ cells with virus expressing EVI1 and control vector. The expression of EVI1 and NGN1 in CD34+ cells will be determined by real-time polymerase chain reaction (PCR).

The researchers also have designed five shRNAs targeting different regions of EVI1 gene. The shRNAs were subcloned into pLKO lentiviral vector. Currently, the research team is testing the inhibitory efficiency of shRNAs on EVI1 expression in human AML cell line with high EVI1 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.

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

Principal Investigator: Claudia O. Rodrigues, PhD

Organization: University of Miami

Grant Progress Report: The laboratory 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. While anthracyclines were discovered more than 50 years ago, their cardiotoxic effects 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 this project is to identify early mechanisms involved in anthracycline toxicity that researchers can target 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. This project officially started in April 2019. During the initial period, researchers first focused on expanding their mouse colony to generate enough animals to start the experiments. The research staff was able to perform experiments with male mice, echocardiography studies and collect samples that are currently being analyzed. During this next period, the team plans to perform experiments with female mice and finish the analysis of samples collected in the current period.

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 Robbins, PhD

Organization: University of Miami

Grant Progress Report: Brain tumors are the No. 1 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 tumor protein 53 (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 staff proposed to identify and target components of signaling networks that regulate TRP53 SHH MB viability. 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 preclinical proof-of-concept data that targeting these novel regulators will reduce MB growth.

Specifically, researchers proposed two aims: identify novel regulators of glioma-associated oncogene 2 (GLI2) required for TRP53 mutant SHH medulloblastoma viability and identify regulators of tumor-propagating cell viability in TRP53 mutant medulloblastoma.

During the last two months, the research team has shown that one of the prioritized candidates, Ubiquitin-like protein containing PHD and RING finger domains 1 (UHRF1, regulates GLI1 activity in a manner comparable to GLI2 activity. Thus, as UHRF1 is not specific for GLI2, the team will move forward with evaluating other candidates from the small interfering RNA (siRNA) screen. Researchers have also identified a list of microRNA 34a (miR-34a) targets and used bioinformatics to arrange these targets into distinct signaling/biological process pathways. Additionally, a list of signaling pathways enriched within a stemness cell cluster in TRP53 mutant SHH MB tissue has been identified using single-cell sequencing and bioinformatics. By comparing these two lists of candidate pathways, research staff has identified and prioritized those candidates common to both approaches and began to evaluate the role a subset of these candidates play in MB sphere culture self-renewal ex vivo.

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: Brain Marples, PhD

Organization: University of Miami

Grant Progress Report: Total body irradiation (TBI) is associated with acute and chronic renal dysfunction, as well as radiation nephropathy (RN). RN represents a serious late complication after radiation therapies for cancer and after TBI as a preparative regimen prior to allogeneic hematopoietic stem cell transplantation (HSCT). RN is irreversible, and no effective clinical treatments exist to prevent RN or ameliorate radiation associated kidney injury. Podocyte loss, tubular atrophy and endothelial damage have been linked with RN, but the molecular mechanisms governing RN are not known. In cultured glomerular podocytes, the research team discovered that the levels of sphingomyelin-phosphodiesterase, acid like 3b (SMPDL3b) are reduced after irradiation, and this triggers the cellular relocation of cytoskeletal proteins leading to a morphological change that alters podocyte functionality.

In the first quarter of the grant period, research staff initiated selected animal experiments described in Aim No. 1. Wild-type C57 Black 6 (C57BL/6) and podocyte-conditional SMPDL3b-knockout C57BL/6 mice were bred and allocated to treatment groups. Six- to eight-week-old animals were irradiated with 10.5Gy total body irradiation and rescued with strain-donor HCST. Wild-type C57BL/6 animals for fractionated treatments (4x4Gy as proposed; and 2x5.75Gy with 24-hour interval) were allocated to groups and irradiated. Blood and urine have been collected from these samples for the first four-week time point, and the next collection time point will be at 10 weeks.

The four-week samples were processed and cold-stored for future quantification of serum blood urea nitrogen (BUN) and creatinine, and proteinuria and albumin/creatinine urine ratio. Blood and urine samples are batch-processed to allow data for all time points to be quantified at the same time to minimize assay variability. Experiments described in Aim No. 1.1 investigating the mitigation of damage have not yet started. The cell culture experiments described in Aim No. 2 have started, and data has been collected. Wild-type and SMPDL3b-overexpressing cells were grown in cell culture.

Cells were irradiated and harvested, and then nuclei harvested to quantify nuclear lipids species using high-resolution mass spectrometry. The research staff has demonstrated that the total Ceramide-1-phosphate (C1P) concentration in irradiated podocyte nuclei follows a bimodal distribution. Five minutes after 2Gy treatment, C1P levels increased from baseline about 2.5pmol/106 nuclei to 10pmol/106 nuclei, then decreased over the next 60 minutes to about 3pmol/106 nuclei and then increased over the next six-to-12 hours to about 6-7pmol/106 nuclei. A similar dose-response pattern was seen for cells irradiated with 8Gy. However, overexpressing SMPDL3b in the podocyte cells, by molecular manipulation, negated the increase in C1P expression after 2Gy and 8Gy at all time points. Similar data was obtained for ceramide and sphingomyelinase. The data for sphingosine showed a clear dose-response with larger response seen at high doses. Together, these data demonstrate that SMPDL3b expression alters sphingosine-ceramide signaling, and this is manifest as cell death by sphingosine-mediated cellular apoptosis. Data demonstrating the recognition and resolution of DNA double strand breaks (using γH2AX assay) has been collected for wild-type and SMPDL3b-over expressing podocytes. These data demonstrate aberrant radiation-induced DNA

damage repair kinetics when SMPDL3b was overexpressed. The same experiments with glomerular endothelial cells are ongoing.

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 to Treat Osteosarcoma

Principal Investigator: Matteo Trucco, MD; Sulagna Banerjee, PhD

Organization: University of Miami

Grant Progress Report: In the last quarter (April-June 2019), research staff have focused on obtaining Institutional Animal Care and Use Committee (IACUC) and Institutional Review Board approval. These were received in May 2019 and June 2019, respectively.

The research team has obtained the cell lines (K7M2 and MG63.2) for osteosarcoma to conduct primary studies. The first experiments have been to treat these cells with the Carbonic anhydrase IX (CAIX) inhibitor WBI-5111 in vitro to standardize a dose for these cells under hypoxia as well as normoxia. The hypoxia chamber is primed and ready for the experiment. The researchers have started validating the dose of WBI-5111 in vitro to available OS cell lines (SaOS2 and U2OS). This will be repeated at 24 hours and 72 hours and the LC50 will be calculated prior to dosing.

The researchers will validate the dose range of WBI-5111 in the MG63.2 and K7M2 cells in vitro as well before starting the animal study. The cell viability assay will be done after 24, 48 and 72 hours after treatment and a LC50 will be calculated.

An experimental metastasis model will start with the K7M2 cells to study if the WBI-5111 and anti-PD1 antibody prevent metastatic colonization of lungs by osteosarcoma 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.

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

Principal Investigator: Mildred Acevedo-Duncan, PhD

Organization: University of South Florida

Grant Progress Report: Multiple compounds that were identified as potential atypical protein kinase C (aPKC) inhibitors were tested against multiple neuroblastoma (NB) cell lines for their effects on cellular growth and viability (in-vitro). 5-amino-1-(2,3-dihydroxy-4-methylcyclopentyl)-1H-imidazole-4-carboxamide (ICA-1S), 8-hydroxy-1,3,6-naphthalenetrisulfonic acid (ζ-Stat), 2-

acetyl-1,3-cyclopentanedione (ACPD) and 3,4-diaminonaphthalene-2,7-disulfonic acid (DNDA) are the compounds which were tested against NB cells such as BE-2C, BE-M17, and CHP-212. Research staff have previously reported ICA-1S as a PKC-iota specific inhibitor while ζ-Stat was reported as PKC-zeta specific inhibitors based on cell-based assays and virtual screening models. In addition, the research team has previously shown that both ACPD and DNDA are effective against both aPKCs. The main purpose was to determine the involvement of aPKCs in NB progression (cellular growth, differentiation, survival, migration, and invasion) in-vitro based on specific inhibition and thereby develop effective inhibitors for latter applications. These results suggest that specificities of the inhibitors are working as intended. Hence, these aPKCs show promise for treating children with neuroblastoma.

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

APPENDIX P

FISCAL YEAR 2018-2019 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	Licht, Jonathan D.	$ 200,000	$ 61,109.00	$ 138,891.00	5/11/2018	4/30/2021	No	No	Yes
8LA02	University of Central Florida	Fernandez-Valle, Cristina	$ 200,000	$ 61,109.00	$ 138,891.00	6/14/2018	4/30/2021	No	No	No
8LA03	University of Miami	Robbins, David J.	$ 200,000	$ 61,109.00	$ 138,891.00	5/21/2018	4/30/2021	No	Yes	No
8LA04	Baptist Health South Florida	Hall, Matthew	$ 700,000	$ 160,416.63	$ 539,583.37	6/14/2018	4/30/2022	No	No	Yes
8LA05	Florida International University	Azzam, Diana	$ 700,000	$ 160,416.00	$ 539,584.00	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 (ALL) cells which harbor the nuclear receptor binding SET (Su(var)3-9, Enhancer-of-zeste and Trithorax) domain protein 2 (NSD2) activating mutation found particularly in children with relapsed ALL. A screen of a large chemical library of anti-cancer compounds showed that the NSD2 mutant cells were resistant to glucocorticoids (GC) — steroids commonly used to treat the disease — but seemed to remain sensitive to other chemotherapy agents. The researchers believe the glucocorticoid resistance may underlie the relapse of these patients.

The research team found that GC failed to induce genes that kill leukemia cells in NSD2 mutant cells, and that removal of the mutation then allowed the GC to be effective as an anti-cancer activity. The researchers performed studies on the ability of the glucocorticoid receptor (GR) to bind across the genome in NSD2 mutant and wild-type cells and discovered that GR levels are depressed in NSD2 mutant ALL cells. Furthermore, in NSD2 mutant cells the GR fails to bind to DNA and to activate genes in response to GC. The mechanism by which this occurs is being examined by studying the chemical modification state of chromatin, the complex of proteins and histone proteins which package DNA in the cell.

The second part of the proposal aims to find the key gene(s) that cause the aggressive brain invading behavior of NSD2 mutant leukemia. Research staff began by learning how to do gene editing screens in myeloma cell lines that are also dependent on NSD2 for malignant growth. The staff will next be turning to the leukemia cell lines, making a custom library of reagents to disrupt the several hundred genes activated by mutant NSD2 to see which are essential for augmented growth in cell culture and in mice.

There have been no institutional changes to affect the research. Dr. Licht has presented the work at the 2018 American Society of Hematology (ASH) annual meeting and seminars at Yale University, Ohio State University and Thomas Jefferson University. A manuscript describing the work is being prepared and another abstract will be submitted to the 2019 ASH meeting.

Follow-on Funding: Leukemia Lymphoma Society; *The Role of NSD2 Mutation in Therapy Resistance in Childhood Acute Lymphoblastic Leukemia*; Jianping Li, MD (Licht Lab Postdoctoral Associate); $134,000.

Rally Foundation*; The Role of NSD2 Mutation in Therapy Resistance in Childhood ALL*; Jianping Li, MD (Licht Lab Postdoctoral Associate); $50,000.

Collaborations: Cold Spring Harbor Labs in Cold Spring Harbor, New York, on the construction of a library of guide RNAs directed against NSD2 targets; Dr. Lock's lab in Australia, regarding primary human ALL with NSD2 mutation; and the National Center for Translational Science at the National Institutes of Health on drug sensitivity of NSD2 mutant cells.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

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

Principal Investigator: Cristina Fernandez-Valle, PhD

Organization: University of Central Florida

Grant Progress Report: The purpose of this research is to identify two compounds 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 this disorder. Surgical removal of schwannomas causes loss of function of the affected body part or sensation of the impacted nerve. Although schwannomas can grow in any nerve, all NF2 patients have schwannomas on the hearing and balance nerve found within the head (cranial nerve eight). In fact, NF2 patients typically become deaf as young adults. Radiation is not recommended for children as there is an increased risk of developing a malignant tumor in the future. Effective compounds/drugs must target the signaling pathway critical for schwannoma cell growth and survival. Previous work identified phosphoinositide 3-kinase (PI3K) as a major signaling pathway to target with drugs. However, schwannoma cells were found to increase activity of other pathways to compensate for the loss of PI3K activity when exposed to PI3K inhibitors. Therefore, an effective drug treatment for NF2-associated schwannoma will likely require the use of at least two drugs.

The goal during the first 14 months of the project has been to complete the drug screen in multiple model cell lines. A total of 15 inhibitor combinations were conducted. The PI3K pathway was targeted with seven inhibitors which were co-administered at 10 increasing concentration with inhibitors to eight unique compensatory pathways. The screen was conducted in up to five human and one mouse schwannoma model cell lines. The most significant finding was the identification of a highly synergistic combination of drugs that together worked better than alone to decrease growth and/or survival of schwannoma model cells. One of the seven tested PI3K inhibitors synergized very well with one of two inhibitors of p21-activated kinase 4 (PAK4) to reduce growth of human schwannoma model cells. The result was similar in the six cell lines tested, providing strong confidence in the finding. The combination will next be tested in an animal model of NF2 schwannoma. The long-term impact of the work on the health of children with NF2 is to contribute to the identification of: 1) Critical pathways used by schwannoma cells to survive and grow, and 2) Compounds or current cancer drugs that can be repurposed eventually for treatment of schwannomas. The research also contributes to 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 through the Biomedical Sciences Program. The following are receiving training or performing research under this project:

Dr. Berta Victoria, a post-doctoral fellow at the University of Central Florida (UCF), is partially supported by UCF and by this award. She will be conducting the animal drug efficacy study aided by Ms. Rosa Rosario who is a senior technician specializing in animal husbandry and partially supported by this award.

Four undergraduate students at the University of Central Florida in the Burnett School of Biomedical Sciences received or continue to receive research training related to this project. One summer undergraduate student from Orlando who is attending Purdue University is being trained and will continue to work during college breaks and summers. The students are as follows:

- Chad Lindo was trained to do western blots for cell line validation for six months.
- Jackson Nagamoto was trained in the validation of the phenotype of the cell lines used by conducting western blot analysis. He also completed animal use training. He trained for approximately one year and is now in medical school.
- Andrew Tritran began basic training in the laboratory three months ago and will contribute to the next phase of this project to replace Jackson Nagamoto.
- Abdul Allaf will continue working on the project when cell confirmation (quality control) is needed at the beginning of the cell grafting experiments. He will confirm the cells are free of mycoplasma and are merlin-null; thus, the cell population has not drifted and are suitable for injection. He has been training for over one year.
- Amrita Kapat is a summer undergraduate student from Orlando attending Purdue University. She has completed the Collaborative Institutional Training Initiative (CITI) animal training module and has been trained on methods of cell line verification that will be done on the cells to be injected into mice. These include mycoplasma testing, and western blots and immunofluorescence staining to assess for Schwann cell markers and the level of merlin expression compared to wild-type Schwann cells. She began her training in May 2019. She will continue to work during college breaks and summers.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

3. Grant #8LA03: Novel Regulators of SHH-driven Medulloblastoma

Principal Investigator: David Robbins, PhD

Organization: University of Miami

Grant Progress Report: Medulloblastoma (MB) is the most common pediatric brain cancer, about 30% of which is driven by mutations in the sonic hedgehog (SHH) signaling pathway. To date, the majority of small molecule inhibitors that block SHH signaling target the pivotal upstream activator smoothened (SMO), which regulates the levels and activity of the glioma-associated oncogene (GLI) family of transcription factors. One such compound, vismodegib, is Food and Drug Administration (FDA)-approved for metastatic basal cell carcinoma patients and is now undergoing clinical evaluation in MB patients. However, rapid tumor recurrence, due to mutations or inherent resistance, has already been frequently observed in MB patients treated with vismodegib. Such observations underscore the critical need to identify inhibitors that act downstream of SMO, ideally on GLI family members themselves. However, as there are few examples in the clinic of drugs that act directly on transcription factors, the researchers have instead focused on the identification of druggable proteins that in turn regulate GLI activity. Consistent with this goal, the research team provided preliminary data showing that the methylation of GLI proteins can modulate their activity or stability, providing a novel way to regulate GLI activity by targeting

these GLI methyltransferases. The researchers hypothesized that these regulators of GLI activity will provide novel ways to modulate its activity, and to attenuate the growth of SHH driven pediatric MB. The goal of this proposal is to elucidate the mechanism of action by which methylation regulates GLI activity, determine the role such modifications play in regulating MB growth ex vivo and demonstrate their functional roles in vivo using mouse and human derived orthotopic mouse models of MB.

Thus far, the researchers have engineered GLI1 (glioma-associated oncogene homolog 1) analogs with loss of function and gain of function mutations based on newly sequenced methylation sites on GLI1 identified from MB tissue. The team has begun to focus on protein arginine methyltransferases (PRMT) responsible for GLI methylation in vivo and are evaluating various short hairpin RNA (shRNA) and PRMT inhibitors to attenuate PRMT activity. Moreover, researchers have shown that PRMT4 inhibitors decrease MB sphere cultures viability, and that they do so in a manner consistent with reduction of GLI activity. They have used immunohistochemistry to identify PRMT4 and PRMT5 in patient MB tissue. These results show that PRMT4 and PRMT5 are overexpressed in MB tissue relative to the surrounding normal tissue. The research team has also begun to evaluate methylation-specific antibodies for GLI1, which could be used as a biomarker to evaluate GLI1 methylation.

Follow-on Funding: None at the time of reporting.

Collaborations: None at the time of reporting.

Journals: Rodriguez-Blanco J, Li B, Long J, Shen C, Yang F, Orton D, Collins S, Kasahara N, Ayad NG, McCrea HJ, Roussel MF, Weiss WA, Capobianco AJ, Robbins DJ. A CK1α Activator Penetrates the Brain and Shows Efficacy Against Drug-resistant Metastatic Medulloblastoma. *Clin Cancer Res.* 2019 Feb 15;25(4):1379-1388. doi: 10.1158/1078-0432.CCR-18-1319. PMID: 30487124.

Patents: None at the time of reporting.

4. 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, MD, MBA

Organization: Miami Cancer Institute, Baptist Health South Florida

Grant Progress Report: The purpose of this research project is to: measure volumetric and morphometric changes in brain substructures in children following radiation to the brain over time; model the dose-volume relationship for brain changes in response to radiation; and correlate these changes with the incidence of neurocognitive, endocrine and quality of life effects.

Brain tumors are the most common primary solid tumor in children in the United States. Although the majority of childhood brain tumor patients are cured, survivors are at risk of developing significant late toxicities that can affect quality of life, limit school and work success and lead to significant medical and social costs. Radiation therapy is known to contribute to

these risks, but data remains limited as to the normal tissue complication probability of different doses of radiation to the brain and the development of these toxicities.

Since opening, six patients have enrolled and have successfully completed all pre-treatment testing. Six-month follow-up scans and evaluations are coming due for the first cadre of patients enrolled on trial.

Collaborating researchers at participating institutions have worked together to ensure that patients can receive testing on protocol at partner institutions and to improve the quality of data collected from this research. In addition, the research team has helped to ensure patients can receive follow-up testing in a timely and efficient manner close to their primary care teams.

Enrollment continues and data is being collected for patients and family who have consented to participate in this research trial. Thus, preliminary results are not available. However, based on this project, meaningful changes have occurred in the brain tumor programs at participating hospitals, in part because of the increasing awareness of the effects that radiation therapy has on brain tumor survivors and the diagnostic tools available to track these changes. The utilization of neuropsychology assessments and neuroendocrine testing have increased significantly after treatment in adult brain tumor patients at the researchers' institution. In addition, a proposal has been generated to systematically test and track these important late effects in all brain tumor patients treated with curative radiation therapy at Miami Cancer Institute. This clinical trial may help better understand anatomic and functional changes that occur in childhood brain tumor survivors after radiation therapy. As an unanticipated consequence of this grant, the research team reports that this project may cultivate similar research initiatives that may help to better understand these important effects in adult brain tumor patients in the future.

Follow-on Funding: Miami Cancer Institute; *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*; Matthew Hall, MD; $100,000.

Collaborations: Radiology (Miami Cancer Institute, Miami, FL) -- Determined optimal reports/measurements to be collected from MRI Volumetric imaging (Neuroquant) examinations

Neurosurgery (Nicklaus Children's Hospital, Miami) – Helped to determine optimal reports/measurements to be collected from MRI Volumetric imaging (Neuroquant) examinations and quality assurance of brain substructure volumes

Neuropsychology (Nicklaus Children's Hospital and Miami Cancer Institute, Miami) – Collaborated to streamline Neurocognitive assessments and protocol-specified questionnaires

Radiology (Nicklaus Children's Hospital, Miami) – Implemented changes in MRI protocols to enable testing at Nicklaus Children's Hospital of the required MR sequence needed to perform Neuroquant analysis

Medical Physics (Miami Cancer Institute, Miami) – Collaborated with Radiation Oncology PIs to methodically collect and record dosimetric data from radiation therapy plans that can be used for assessment of anatomic and morphometric changes in the brain, the primary study objective.

Journals: None at the time of reporting.

Patents: None at the time of reporting.

5. 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 the research team's robust high throughput ex vivo drug sensitivity testing (DST) and combining it with mutation analysis. The main outcome of the study is the proportions of the patients whose treatment was guided by the personalized medicine approach. From July 1, 2018, to June 30, 2019, the researchers received Institutional Review Board (IRB) approval including modifications, signed institutional contracts, held team meetings, performed a trial run of samples and consented and recruited a total of six patients with recurrent/refractory cancers.

Most importantly, the research team optimized and successfully performed its drug sensitivity assay on six patients with recurrent leukemias, metastatic osteosarcomas, rhabdomyosarcomas and lung cancers. Of those six, researchers were able to perform DST and mutation profiling on five. These patients showed vastly different responses to the 107 Food and Drug Administration (FDA)-approved compounds used in the screen. Indeed, none of the compounds showed activity in all the patients, and more than half of the evaluated compounds were not active in any of the patients.

Research staff is currently assessing patients' clinical response to DST-guided treatment. One patient with recurrent leukemia and guided by DST treatment has already achieved complete response. Since the objective is to recruit four patients a year, the research team has successfully achieved target accrual for the year. Overall, researchers have confirmed the feasibility of this methodology in children to identify candidate agents with clinical potential and would like to continue to implement this novel and personalized approach to assess clinical response and disease-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.